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THE Committee appointed by the *Royal Society* to direct the publication of the *Philosophical Transactions* take this opportunity to acquaint the public that it fully appears, as well from the Council-books and Journals of the Society as from repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries, till the Forty-seventh Volume; the Society, as a Body, never interesting themselves any further in their publication than by occasionally recommending the revival of them to some of their Secretaries, when, from the particular circumstances of their affairs, the *Transactions* had happened for any length of time to be intermitted. And this seems principally to have been done with a view to satisfy the public that their usual meetings were then continued, for the improvement of knowledge and benefit of mankind: the great ends of their first institution by the Royal Charters, and which they have ever since steadily pursued.

But the Society being of late years greatly enlarged, and their communications more numerous, it was thought advisable that a Committee of their members should be appointed to reconsider the papers read before them, and select out of them such as they should judge most proper for publication in the future *Transactions*; which was accordingly done upon the 26th of March, 1752. And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them: without pretending to answer for the certainty of the facts, or propriety of the reasonings contained in the several papers so published, which must still rest on the credit or judgment of their respective authors.

It is likewise necessary on this occasion to remark, that it is an established rule of the Society, to which they will always adhere, never to give their opinion, as a Body, upon any subject, either of Nature or Art, that comes before them. And therefore the thanks, which are frequently proposed from the Chair, to be given to the authors of such papers as are read at their accustomed meetings, or to the persons through whose hands they received them, are to be considered in no other light than as a matter of civility, in return for the respect shown to the Society by those communications. The like also is to be said with regard to the several projects, inventions, and curiosities of various kinds, which are often exhibited to the Society; the authors whereof, or those who exhibit them, frequently take the liberty to report, and even to certify in the public newspapers, that they have met with the highest applause and approbation. And therefore it is hoped that no regard will hereafter be paid to such reports and public notices; which in some instances have been too lightly credited, to the dishonour of the Society.

I—THE LIFE HISTORY OF SOME MARINE PLANKTON DIATOMS

By F. GROSS, *Marine Biological Laboratory, Plymouth*

(Communicated by E. J. Allen, F.R.S.—Received 12 December 1936—Read 29 April 1937)

[PLATES 1–4]

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INTRODUCTION

The diatoms are divided into two big groups, the Pennatae and the Centricae. The first group consists mainly of bottom forms which are either attached or show free movement, whereas the non-motile, suspended Centricae form the main bulk of the planktonic diatoms. The present work is concerned with the second group only.

A knowledge of the essential structure of the cell, which is very similar in both groups, is of great importance for understanding the life history of the diatoms. The cell walls consist of two parts which overlap each other like the two halves of a pill box; the valves forming the top and bottom, the connecting bands or girdle the sides of the box. In the course of cell division the two halves of the cell wall become separated and each daughter cell forms a new valve and new connecting bands to fit into the old girdle. Owing to the silicification of the cell membranes, growth is possible only along the perivalvar axis, the longitudinal axis of the "box". Consequently the diameter of one cell is slightly smaller than that of its sister cell, i.e. by twice the width of the cell wall. In a population this peculiar mechanism of cell division leads to a continuous decrease of the average cell size.

As early as 1869 MACDONALD and PFITZER (1871) established the existence of a regulatory process which counteracts the decrease of cell size by the formation of special cells which are capable of growth, the auxospores. Later work, notably by KARSTEN, KLEBAHN and more recently by GEITLER (1932), produced quite a clear

picture of the life history of the Pennatae. In most forms the production of auxospores is preceded by the fusion of gametes, these representing the only haploid phase in the life cycle of the Pennatae.

Our knowledge of the centric diatoms is far less satisfactory. The life cycle is complicated by the occurrence of resting spores and microspores as well as of auxospores.

Resting spores have been frequently found in many species. They are regarded as resting stages which sink to the bottom and persist through unfavourable periods (GRAN 1902). From the literature it appears that the sequence of their formation and germination has so far never been observed.

Microspores were observed by several authors (see p. 38) and regarded as gametes or as asexual reproductive cells. It must be pointed out that none of the authors has actually observed the fusion of microspores or the development of the zygote. According to GEITLER (1932) this fact might perhaps find its explanation in observations recorded by WENT (1924; see also his letter to GEITLER (GEITLER 1932)). WENT studied the plankton on a journey across the Atlantic. One day the only diatoms to be found were two forms of *Chaetoceros* of which the larger was full of microspores while the smaller cells were surrounded by flagellated swimmers of similar size to those within the larger cells. WENT suggested that the microspores of the larger form were microgametes which fertilized the female cells of the smaller type.

The auxospore formation in centric diatoms was formerly generally regarded as a simple growth phenomenon of the protoplast, not connected with either a sexual process or any nuclear changes. Recently, however, PERSIDSKY (1929, 1935) found first in *Chaetoceros boreale* and *Ch. densum*, later in *Melosira varians*—the same species in which SCHMIDT described microspore formation—that the formation of auxospores is connected with an autogamic sexual process.

From this short review it can be seen that with regard to the life history of centric diatoms the views of different authors differ quite fundamentally. When I came to the Plymouth Laboratory last year I started culturing planktonic diatoms in the hope that a study of pure cultures might clear up some of the contradictory statements in the literature.

Apart from its purely botanical interest such a study appeared desirable from the point of view of marine biology. Plankton diatoms are of great importance as food in the sea. Their distribution and their seasonal and yearly variation in abundance, in connexion with the corresponding hydrographical conditions and chemical changes of the sea water, have been the subject of many investigations. However, owing to our incomplete knowledge of their life cycle those surveys were mainly concerned with the vegetative cells, and references to auxospores are very rare. The resting spores, evidently of some importance for the understanding of the seasonal variations in the abundance of diatoms in the sea, have been frequently found in plankton samples by various workers, but so far there does not exist any experimental evidence concerning the conditions responsible for their formation and germination.

The present work has been made possible by a grant from the Academic Assistance Council and a scholarship from the Marine Biological Association. I wish to express my sincere thanks to Dr. E. J. ALLEN for his kind interest and the encouragement I received from him throughout the work, and for the excellent facilities of the Plymouth Laboratory which he placed at my disposal. My thanks are also due to Miss M. V. LEBOUR who identified most of the diatoms I used, and to Dr. E. J. ALLEN, Dr. S. KEMP, and Mr. F. S. RUSSELL who read the manuscript of this paper and made various corrections and valuable suggestions for alterations.

CULTURE METHODS

One of the reasons why the life history and physiology of the pennate diatoms is so much better known than that of the Centricae may be found in the fact that the former are more resistant and easier to culture than the latter. The classic work on culture conditions for centric diatoms was done by MIQUEL (1892-7), ALLEN and NELSON (1910) and ALLEN (1914). The technique used by these authors was either to pick out individual diatoms under the microscope and to place them in a sterile culture medium (MIQUEL's or ALLEN's modification of it) or to dilute a small quantity of a plankton sample containing diatoms in a certain amount of culture fluid and to subdivide this into a number of culture flasks. In one or the other culture one diatom species only would develop. Another method employed by ALLEN was to start a raw culture with one or two drops of plankton diluted in about 250 c.c. of sterile culture medium which was distributed over a number of Petri dishes. If these are left undisturbed for a few days colonies of different diatoms will develop at different spots on the bottom of the dish. These were picked out with pipettes and transferred into culture flasks.

I used a different technique which gave very satisfactory results. As culture medium FÖYN's "Erdschreiber" (FÖYN 1934) has been used. This is a modification of SCHREIBER's medium (SCHREIBER 1927), soil extract being substituted for distilled water. It consists of

NaNO_3	0.1 g.	Soil extract	50 c.c.
Na_2HPO_4	0.02 g.	Sea water	1000 c.c.

The diatoms were isolated from plankton samples by picking out single individuals with fine pipettes. It is essential to wash the isolated cells by passing them through three or four sterile water samples in watch-glasses. Next day the washing is repeated once or twice and the cells left for a few days in a watch-glass, well covered with a glass plate. If the diatoms reproduce satisfactorily a sample may be washed after a week or so and transferred into a Petri dish. All culture dishes, flasks, and pipettes were thoroughly cleaned and sterilized before use.

The cultures were kept at room temperature and placed near a window facing north. During the winter months a 100 W lamp hanging at a distance of about 1 m. was used to increase the light intensity during the day.

Subcultures were made every 2–3 weeks and inoculated mostly with 10–100 cells.

Bacteria-free cultures were not aimed at and no special bacterial tests made. Since the cultures were frequently examined, sometimes two or three times a day, and a large surface of the watch-glasses and Petri dishes was thus exposed to the air, the occasional appearance of mostly harmless bacteria was to be expected. On the whole I was satisfied if there was no obvious sign of bacterial contamination. A more detailed account of the culture methods has been published elsewhere (Gross 1937).

DITYLUM BRIGHTWELLI (West.)

Most observations and experiments were done on this neritic species (fig. 1, Plate 1). The cells are elongated, usually solitary. In young, rapidly growing cultures they sometimes form short chains of 4–12 cells. The valves possess in the centre a strong straight hollow spine, and between the centre and the edge a collar of short, mostly curved spines. The species is described as being prism-shaped with rounded angles to nearly cylindrical. It may be noted that in cultures the progeny of prism-shaped cells gradually become cylindrical. On the other hand, the majority of cells developed from auxospores in a culture started with a single cylindrical cell were prism-shaped but also included forms with round, flat, elliptical and tetragonal valves. It seems therefore likely that the subspecies distinguished by taxonomists (see Hustedt 1930) forma *biangulata* and forma *tetragona*, with oval and tetragonal valves respectively, represent variations of the same type.

In cultures with many cells produced from auxospores there also occur forms which show a close resemblance to what is described as *Ditylum sol* GRUN., the marginal ridge being more undulated, with several longitudinal lines showing up in girdle view, without the collar of small spines and with triangular valves, the sides of the triangle being slightly concave. As the distinction of this species is almost exclusively based on those characters it is not unlikely that what has been regarded as two species of *Ditylum* are again modifications of one species. This question should, however, be settled by someone with better taxonomic qualifications. I mention the above facts to show that even in pure lines there exists a considerable range of variability affecting a number of characters of systematic importance.

(a) Cell division

The cells of *Ditylum* grow in a longitudinal direction until they reach a certain maximum size which is rather uniform within a culture, but which may differ markedly from subculture to subculture (see p. 27). The division of the nucleus and protoplast then takes place and the new valves are formed inside the parental shell before the

separation of the daughter cells (fig. 1*b*, *d*, Plate 1). After separation the new valve lies at first well within the old girdle (fig. 1*c*, Plate 1) and is gradually being pushed forward out of it (fig. 1*a*, Plate 1).

The division of the protoplast takes about 30 min. Some stages of the division followed in single cells are shown in figs. 2 and 3 (Plate 1). The main bulk of protoplasm is concentrated near the centre of the cell, usually connected by two protoplasmic strands with the centre of the valves (fig. 2*a*, Plate 1). The division begins at the periphery of the cell and proceeds towards the centre. It is unlike the division of other Protozoa and Algae in that the incision is very broad from the beginning. The protoplast takes the shape of a dumbbell (fig. 2*b*, Plate 1), the prospective daughter cells being connected with each other by a protoplasmic bridge. This connexion becomes gradually very fine and eventually gets resorbed (figs. 2*c-e*, 3*a-c*, Plate 1), leaving a wide empty space between the daughter cells. The way in which the protoplasm is drawn out in the course of division, and the fact that the protoplasts of the two daughter cells together fill a space far smaller than the original parental one 20 or 30 min. before, suggest the existence of a strong bipolar tension prior to and during the division. The new central spines are formed a short time after cell division (fig. 2*f*, Plate 1, 8 min.; fig. 3*e*, Plate 1, about 40 min.) and most probably before the formation of the new valves. During their growth the spines are soft and whenever they touch another object—the opposite spine (fig. 1*d*, Plate 1) or the primary valve (fig. 7*b*, *c*, Plate 2)—they are liable to become bent. The growth of the spines takes $\frac{1}{2}$ hr. at most. The separation of the cells, however, takes place several hours later, after the formation of the small spines and the silicification of the new valves.

(*b*) Nuclear division

Since the first detailed investigation of the nuclear division in *Surirella* by LAUTERBORN a number of other pennate diatoms have been studied cytologically (see FRITSCH 1935). The extranuclear origin of the spindle as described by LAUTERBORN has not been confirmed, and apart from the existence of centrosomes in some pennate diatoms the mitotic division does not seem to be fundamentally different from other Algae. However, IKARI (1923) has described an extranuclear spindle similar to that of LAUTERBORN in *Surirella* in the centric diatom *Coscinodiscus subbulliens*, and SCHMIDT (1927, 1928, 1929, 1931) has recorded a number of extremely peculiar cytological features in *Biddulphia sinensis* and other centric forms. His methods and interpretations were strongly criticized (GETTLER 1931; v. CHOLNOKY 1933), and some of his statements were recently withdrawn (SCHMIDT 1933).

v. CHOLNOKY (1933) studied mitosis in *Melosira arenaria* and established a perfectly normal origin of chromosomes and spindle. In this respect I was able to confirm his results, but in some details the nuclear division in *Ditylum* differs from that of *Melosira*. A short account may therefore be of some interest.

For the cytological study of the plankton diatoms I have been using the quick and simple method of acetic carmine. This preserves and stains simultaneously and has in recent years been extensively used in plant cytology and work on *Drosophila*. It gives good results with diatoms with not strongly silicified membranes as in *Ditylum* and *Chaetoceros*. It is prepared by dissolving carmine to saturation in hot acetic acid—1 part distilled water, 1 part acetic acid—and filtering. A number of cells with as little fluid as possible are placed on a glass slide in a drop of acetic carmine and heated for a moment over a small flame. The drop is then covered with a cover-slip, a ring of hot vaseline put round the edge and the preparation is ready for use.

Dividing cells were found at any time of the day in cultures that were not overcrowded; on the average perhaps more divisions take place in the early morning.

The nucleus lies within a central protoplasmic area which is suspended in the cell and connected with the protoplasmic layer inside the membrane by means of two or more protoplasmic strands. Before or during division of *Ditylum* cells the central protoplasm with the nucleus moves towards the cell membrane. In broad cells all mitotic figures were found to lie near the side wall of the cell, the girdle, in continuity with the protoplasmic layer where the numerous small chromatophores are situated. In cells of smaller diameter this movement does not seem to take place as regularly as in broad ones. v. CHOLNOKY has found a similar movement in *Melosira* cells: the nucleus is drawn by the protoplasmic strands towards one of the valves and sometimes becomes slightly deformed.

The resting nucleus appears finely granulated and interspersed with somewhat stronger stained chromatic granules (figs. 4a, 5a, Plate 1). In the centre lies a single nucleolus, of almost homogeneous appearance, not vacuolized as in *Melosira arenaria*. In the prophase it disappears; the whole body of the nucleus is filled with numerous chromatic threads. The individual limits of the chromosomes are indistinct—a stage which formerly might have been interpreted as a continuous spirem (fig. 5b, Plate 1). The next figure (5c) shows a well-developed central spindle, while the chromosome threads have undergone little change. Centrosomes or corresponding structures appear to be absent throughout the mitosis. In the late prophase (fig. 4b, Plate 1) the shape of the individual chromosomes is becoming distinct. Their structure is that of long fine threads of chromomeres, the connecting substance between these being almost unstained. Some evidence for the assumption that the spindle is of intranuclear origin is produced by stages like fig. 5d (Plate 1), where the spindle is fully developed and the nuclear membrane is more or less intact. The chromosomes are here on the way towards the equator, their proximal parts more advanced than their distal ends. It may be noted, however, that in most cases the nuclear membrane is already absent or at least not visible at earlier stages.

In metaphase (fig. 4c, d, Plate 1) the attachment of the chromosomes to the spindle seems to be terminal. The chromosomes are stretched in almost one plane. They are very delicate and not appreciably more condensed since the prophase. Exact counting

of their number is impossible; it may be roughly estimated at 40–50. The spindle has the shape of an ellipsoid rather than that of a cylinder.

The anaphase movement must take place with great rapidity. Among many hundreds of division stages not a single middle anaphase was found. In the late anaphase (fig. 5*e*, Plate 1) the chromosomes are lying more or less parallel to the long axis of the cell. The telophase (figs. 4*e*, 5*f*, Plate 1) is perfectly normal, the chromatine structure resembling that of the prophase. A spindle remnant remains visible throughout the restoration of the resting nucleus (fig. 4*f*, Plate 1). The nucleolus appears at a very late stage (fig. 5*g*, Plate 1); this figure also shows the outline of the dividing protoplast).

On the whole the mitotic figure—chromosomes, nucleolus, spindle—are much more delicate in *Ditylum* than in *Melosira*. Their structure, however, is essentially the same. In *Melosira* the nucleolus persists through a long period of the prophase until the formation of the spindle, and it reappears after division in an earlier telophase stage. There is no “spirem” stage; in the early prophase the chromosomes appear in the shape of individual threads in smaller number than in *Ditylum* and the spindle develops very late in the prophase. At metaphase the chromosomes are much more condensed, shorter and thicker than in *Ditylum*, and form a dense and compact ring around the spindle as in the Pennatae. On the whole the mitosis of *Ditylum* does not seem to differ from that of other Algae, higher plants and animals in any essential character.

(c) *Secondary valves*

The formation of new valves is not entirely dependent on cell division. In dense cultures of *Ditylum* one can frequently find empty valves at the bottom of the dish. They originate from cells of the type represented in fig. 6*a* (Plate 2). Within the old shell a smaller cell with a new hypotheca has been formed. The formation of such cells with secondary valves could be followed when single cells were isolated.

On 25 November a cell which had undergone division on the same day was isolated in a watch-glass (fig. 7*a*, Plate 2). Next day both daughter cells possessed secondary valves. One of them is drawn in fig. 7*b* (Plate 2). The new spine has apparently touched the old primary valve during its growth and was consequently bent. This cell was again isolated. Next day it showed considerable growth in length, the old valve being pushed off but still hanging on the spine of the new secondary valve. (When transferred with a drop of culture fluid on a cavity slide to be drawn under the microscope the primary valve came off. It has been drawn in about the same position as it originally lay in the watch-glass.) Subsequently the cell showed normal division.

Such cases have been repeatedly observed in various lines. There is reason to assume that the formation of secondary valves is caused by a preceding contraction of the protoplast. A cell like figs. 6*a*, or 7*b* (Plate 2) must have been covered inside the whole membrane with a protoplasmic layer containing the chromatophores. Owing to the contraction of the protoplast towards the epitheca a condition is created similar to

that after the division of the protoplast and a new valve is formed. Actually a contraction of the protoplast could several times be observed when placing cells on glass slides for microscopic examination. It is a very rapid process, in these cases probably caused by mechanical irritation. This, however, does not explain the occurrence of cells with secondary valves in cultures which at least for some time had been quite undisturbed.

In crowded cultures several valves can be formed successively by a single cell. Up to eight have been observed. Fig. 6b (Plate 2) is a drawing of a resting spore with five valves attached to it.* The original protoplast has contracted four times and each time it has formed a new valve. Eventually a resting spore was formed.

Cells with secondary valves have also been found in nature. MEUNIER (1915) gives a figure of a *Ditylum Brightwelli* cell containing a secondary valve and describes it as "Phase de division avortée par résorption de l'une des deux cellules filles et le maintien d'une seule cellule jeune au sein de la matricule".

PAYNE (1925) studied plankton from Hong Kong which contained a great number of *Ditylum sol.* Among them he found hundreds of forms of *Ditylum* "in which the frustule developed only one new frustule; the second, oldest valve being barren, and pushed off as the newcomer became fully grown". "No secondary frustule showed any indication of the development in it of a third frustule. . . ." PAYNE suggested that the secondary frustules may be resting spores of *Ditylum sol.*

HUSTEDT (1930) found similar stages of *D. Brightwelli*. He regards them as cells with incomplete division, having formed at the end of the mother cell one young daughter cell while the formation of the sister cell has been omitted.

My observations on cultures show that the interpretations of these authors were not correct. It is obvious that the formation of secondary valves does not represent an incomplete, interrupted division, nor are two daughter cells formed, one getting resorbed, as MEUNIER suggested. No nuclear division is connected with it, and the protoplast never shows any sign of division. Moreover, as has been stated further above (p. 4), the cells of *Ditylum* grow to a certain maximum length before cell division takes place. A comparison of the cell length in fig. 6a with fig. 1b (cells from the same subculture!) and of fig. 7b with fig. 7a (Plate 2) shows that the cells with secondary valves were far from having reached the size of cells undergoing division. No daughter cell has been formed, but the same protoplast has after its contraction produced a new valve.

From the following section it will be seen that resting spores of *Ditylum* are quite different from secondary frustules. It may well be that some of the factors responsible for resting spore formation also cause contraction of the protoplast and subsequent formation of secondary valves. This would explain why sometimes the formation of resting spores is preceded by that of secondary valves (fig. 6b, Plate 2).

* The resting spore was lying at the bottom of the dish with the row of valves directed upwards, so that on the camera lucida drawing the length of the valves appears shortened.

The formation of secondary valves bears some resemblance to the "moulting" of thecate dinoflagellates; a phenomenon which I have repeatedly seen in dense cultures of *Prorocentrum micans*. It is also similar to the formation of "internal valves" frequently recorded in pennate diatoms (HUSTEDT 1930; GETTLER 1932), in which, however, both valves are newly formed, a more or less complete shell within the old parental one.

(d) *Resting spores*

The first culture of *Ditylum* (line A) was started on 1 October 1935 with twenty cells taken from a plankton sample. On 5 October the first subculture was made, and 16 days later many resting spores were found in it. These are more or less spherical bodies of very dense structure lying within the original cell membrane (figs. 8a, 9a, Plate 2). Some had developed a strong silicious membrane so that neither the chromatophores nor any other structural details could be seen under the microscope (fig. 6b, Plate 2). Each cell contained one resting spore.

On 7 November a smaller number of resting spores was found in the 15 days old subculture 2 together with some auxospores; there were also many cells with a shrunken protoplast stretching from valve to valve but, under low power, apparently disconnected from the side wall of the cell. The explanation of these peculiar cells appeared in subculture 3 which was started on 1 November. On examination after 10 days very many resting spores were seen in the morning. When, however, the culture was again examined in the afternoon to start a fresh subculture not a single resting spore could be found. All had germinated during the day into perfectly normal vegetative cells. The same striking phenomenon was observed next day: 10 a.m., culture full of resting spores; 1 p.m., few resting spores, very many germinating resting spores, i.e. cells with a long, tube-shaped protoplast; 6 p.m., two resting spores found among many thousands of vegetative cells.

As shown in Table I, this "rhythmic" process of daily formation and germination of resting spores continued until 22 November, when few spores were recorded ungerminated at 6 p.m. After 26 November all resting spores persisted, and no further germination took place in the culture. The number of cells was estimated at 120,000, or 3000 per c.c. of culture fluid, of which about 70 % had formed persistent resting spores.

Table II shows the conditions in subculture 4. Here the first resting spores appeared after 13 days. During the next 10 days a great number of resting spores were formed overnight and germinated in the course of the day (some exceptional days will be discussed later; see p. 18). After that few spores were found ungerminated in the evening, and when the culture was 33 days old they persisted in great numbers without germination.

Several parallel cultures showed very similar behaviour. In subculture 5 the first appearance of resting spores was rather late, 18 days after the subculture was made

F. GROSS ON THE LIFE HISTORY OF

TABLE I—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 3

Date	Age of culture in days	Room temperature in ° C.		Illumination integral in kilolux-hours	Number of resting spores	
		Min.	Max.		10 a.m.	6 p.m.
Nov. 11	10	8.9	11.4	129	Very many	0
12	11	10	15	118	Very many	0
13	12	8.9	15.6	94.7	Very many	0
14	13	10.6	15.6	47.4	Few	0
16	15	10	—	107	Very many	0
18	17	8.3	15.6	106	Very many	0
20	19	10	15.6	49.6	Many	0
21	20	10.6	17.8	124	Many	0
22	21	10.6	16.7	77.7	Very many	Few
23	22	10	14.4	106	Very many	Few
24	23	8.3	13.3	—	Many	Few
25	24	6.7	14.4	71.6	Many	—
26	25	10	13.9	63.9	Many	Many
27	26	—	13.9	56.5	Very many	Very many

TABLE II—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 4

Date	Age of culture in days	Room temperature in ° C.		Illumination integral in kilolux-hours	Number of resting spores	
		Min.	Max.		10 a.m.	6 p.m.
Nov. 20	9	10	15.6	49.6	0	0
24	13	8.3	13.3	—	Few	0
25	14	6.7	14.4	71.6	Many	0
26	15	10	13.9	63.9	Many	0
27	16	—	13.9	56.5	Many	0
28	17	11.7	16.7	37.3	0	0
29	18	12.2	16.1	96.5	Very many	0
30	19	11.7	—	37.3	0	0
Dec. 3	22	11.7	—	33.7	Many	—
4	23	7.2	16.7	83.5	Very many	Few
6	25	9.4	16.1	49.7	Few	Few
7	26	10	15	62.2	Very many	—
9	28	10	14.4	84.1	Few	Few
14	33	7.2	15	70.4	Very many	Very many

(Table III). Four days later many resting spores were formed overnight and germinated in the daytime. When the culture was 29 days old the resting spores persisted.

Resting spores were formed in cultures of *Ditylum* irrespective of the size of the cells, by very narrow ones as well as by cells which had developed from auxospores a few

TABLE III—OCCURRENCE OF RESTING SPORES IN SUBCULTURE 5

Date	Age of culture in days	Room temperature in ° C.		Number of resting spores	
		Min.	Max.	10 a.m.	6 p.m.
Dec. 14	16	7.2	15	0	0
16	18	7.2	—	2	0
18	20	10.6	—	10	0
20	22	7.2	14.4	Many	0
27	29	6.7	13.9	Very many	Very many

days before. The only difference was the larger size of the spores. Cultures of broad cells originated from auxospores also showed formation of resting spores overnight and their germination during the day.

The germination of resting spores can be followed in single cells (figs. 8-11, Plate 2). The repeated pipetting to and fro from the watch-glass where the cell was isolated to a cavity slide for drawing under the microscope slows the speed of germination down considerably. While the spores in the Petri dishes, except the persistent ones, complete germination in the evening of the same day, the isolated cells still showed some contraction of the protoplast in the evening (figs. 8*d*, 10*c*, 11*c*, Plate 2), and only regained the appearance of vegetative cells on the following day. The process is, however, not altered.

The first sign of germination is a slight elongation of the spore (fig. 8*b*, Plate 2). Fine protoplasmic filaments similar to very delicate pseudopods connect the spore with the old cell membrane. It may be noted that in most of the cases observed the first connexions sent off by the spore run towards the centre of the valve opposite the insertion of the long spine. Most of the filaments send off branches a short distance from the membrane or form a network. The elongation of the protoplast continues and the "pseudopods" become more conspicuous. Sometimes chromatophores could be watched slowly passing along a protoplasmic strand towards the membrane. Gradually the expansion of the protoplast is completed and the cell takes on its normal appearance: viz. with the inside of the thecae covered with a protoplasmic layer containing the chromatophores and in the centre the main body of protoplasm containing the nucleus, suspended on a number of protoplasmic strands.

The germination of individual spores shows some differences in detail. In narrow cells their elongation proceeds mostly in the direction of the long axis of the cells (fig. 8, Plate 2). In broader cells the protoplast stretches more irregularly throughout the inner space. Some germinating spores are suspended at or near the centre of the shell by fine or more solid "pseudopods" (fig. 11, Plate 2), others come into broader contact with one of the valves and from there send off connexions to the rest of the shell (fig. 10, Plate 2). Fig. 9 (Plate 2) shows that even in cells shortly after division where the volume of the resting spore does not greatly differ from that of the original cells germination starts by the sending off of protoplasmic filaments, and does not merely consist of a gradual expansion of the body of the spore as one might have expected.

It is of some interest to note the differences between the germination of the resting spores represented in figs. 10 and 11 (Plate 2). These were sister cells connected with each other like those on fig. 1*d* (Plate 1) and only separated in the course of isolation from the culture. In fig. 10 (Plate 2) the spore expands along the valve taking a more or less oval shape, and after a few hours (3.30 p.m.) shows inside the structure of the vegetative cell, with a central mass of protoplasm with strands extending to the periphery. The germination appears less "amoeboid" than in fig. 11 (Plate 2).

However, the anchoring of the germinating spore to the shell by means of fine protoplasmic processes is a characteristic feature observed in all cases.

The germination described above was observed on resting spores which were isolated in watch-glasses with fresh culture medium; these would, however, have also germinated in the original cultures. In these the resting spores at the time of isolation were formed overnight and germinated in the course of the day. To induce germination of persistent resting spores which develop a silicious membrane and never show any sign of development in the culture, it is necessary to transfer them into fresh culture medium. The germination takes 2–10 days or even longer and proceeds in exactly the same manner as described above.

In old overcrowded cultures a considerable number of resting spores could be found outside the parental shells. When transferred into new culture medium they only expanded in volume, but as yet the formation of valves has not been observed.

The formation of resting spores could not be followed in single cells since isolation inhibits and usually reverses the process. But in cultures with cells slowly undergoing formation of resting spores one can easily study all stages of this process. Fig. 12 (Plate 2) gives four stages and shows that the formation of resting spores is almost exactly the reverse of germination. The protoplast becomes detached from the shell and contracts gradually into a compact, more or less spherical body. At first it remains connected with the thecae by means of a number of fine, partly branched, protoplasmic filaments which become resorbed in the course of the contraction. Occasionally some chromatophores could be observed passing along these filaments towards the central body of protoplasm. And again in most cases, the strongest connexions were those leading to the centres of the valves, the point of insertion of the long spine (fig. 12*b, c*, Plate 2). These also were the last to be resorbed.

Resting spores were frequently found in tow nettings. In autumn and winter after complete absence of *Ditylum* from the plankton a few resting spores were usually found together with vegetative cells and cells like those represented on fig. 13*a, b* (Plate 3), which might be stages of either the formation or germination of resting spores. Since their appearance coincided with that of vegetative cells of *Ditylum* after periods of absence from the plankton they probably were germinating resting spores. When isolated and placed in culture medium they developed in less than 24 hr. into normal vegetative cells.

In an attempt to analyse the factors responsible for the formation of resting spores let us begin with the observations recorded at the beginning of this section and in Tables I–III. The outstanding facts are as follows: (1) No resting spores were formed before the cultures reached the age of 9, 13, and 18 days respectively. During this period the cells reproduced actively and the Petri dish cultures reached a density of 30,000–50,000 cells in about 35 c.c. of fluid, i.e. about 1000 cells per c.c. of culture medium. (2) There followed a period of 9–15 days with formation and subsequent germination of a great number of resting spores. The reproduction of the cells not

undergoing spore formation continued and the culture became very dense. (3) Finally the resting spores developed a stronger silicious membrane and persisted. The cultures were crowded with resting spores and sometimes also with auxospores. The remaining vegetative cells became dark brown and did not divide any more.

Taking first the second phenomenon it appeared possible that the differences of the room temperature by day and night were the causes of the successive formation and germination of the resting spores. In Tables I–III the minimum and maximum temperatures are given: the differences are quite considerable. At night the temperature went down sometimes as low as 6.7°C ., in the morning it was usually about 10° and in the course of the day it rose to 14 – 16° . I first thought that the temperature must drop below 10° to be effective. On 14 November (Table I) comparatively few resting spores had been formed; the minimum temperature was 10.6° . Again in another culture no resting spores were found on some days (28, 30 November; Table II) when the minimum temperature was 11.7° . However, very many were found on 29 November in spite of the high minimum temperature of 12.2° . Therefore probably another factor perhaps in conjunction with low temperature was affecting the cultures.

That low temperature could not be the only factor responsible for resting spore formation was clear from the beginning, because, as mentioned above, young cultures under otherwise the same conditions did not produce resting spores. Subcultures 3 and 4 were kept in the same place near the window, one Petri dish above the other. Subculture 4 did not produce a single resting spore from 11 to 23 November, while during the same period subculture 3 was crowded with them (see Tables I and II). Thus apparently the age of the culture was of importance, in that perhaps some changes were produced in the culture medium by the reproduction of the diatoms to a certain number of cells per c.c. of fluid.

To test this a number of simple experiments were made. One, ten, twenty, and 100 cells were isolated on various days into watch-glasses from cultures which produced very many resting spores overnight. The watch-glasses were placed on top of the Petri dish culture or very near it. Not a single resting spore was found either the next or the following day, while in the original culture more than half of the cells underwent spore formation.

Under these conditions—cultures with few cells in fresh culture medium—low temperature and at the same time absence of light (ice-box) did not seem to have any effect with regard to the formation of spores. On 1 November two resting spores were isolated from subculture 2. They germinated the next day. During the following days they showed a slow reproduction but no resting spores were formed. On 13 November thirty cells were transferred from that culture into a watch-glass and this placed in an ice-box (7.2°C .) for 4 days. No resting spores were formed. In other experiments a few cells were exposed to low temperature of varying degrees for periods up to a week with the same result.

On 18 November, 10.15 a.m., 100 resting spores were taken out of subculture 3, washed, and placed in a watch-glass with about 1 c.c. of fresh culture medium. They germinated in the course of the day in the same way as the resting spores which had remained in the original culture (see Table I). Division took place but no resting spores were formed during the following 4 days. On the 5th, 6th and 7th day three resting spores were seen, on the 12th day nine resting spores (and four auxospores). On the 14th day at 10 a.m. the culture contained 9–10 resting spores and was placed in an ice-box (3.3°C.). At 2.30 p.m., only $4\frac{1}{2}$ hr. later, there were considerably more resting spores of normal appearance, and almost all the rest of the diatoms formed spores of somewhat irregular shape (fig. 14*a*, Plate 3) with the contracted protoplast in the centre and few chromatophores and granules left outside the spore. The culture was left for 2 days in the ice-box and the cells counted. There were:

99 regular resting spores,
852 irregular resting spores,
9 auxospores,
2 vegetative cells,
<hr/>
962

The cells were counted by cautious pipetting from the original watch-glass into a new one. They were left in it with the old culture medium, and after 2 days it was found that all irregular resting spores had taken regular, more or less spherical shape. (A similar process is described further below; see fig. 15, Plate 3.) One irregular spore, isolated into fresh "Erdschreiber", germinated in the course of 5 days (fig. 14, Plate 3). Germination of the other resting spores started only after 10 December, when they were transferred into fresh medium.

In this experiment, with almost 1000 cells in about 1 c.c. of medium, the low temperature was very effective since the resting spores were formed within $4\frac{1}{2}$ hr. instead of several days. It seems likely that the irregular shape of the spores was caused by the very sudden change of the temperature inducing a hasty spore formation. As they mostly took regular shape after the exposure to low temperature had ceased there seems to be no fundamental difference between the two types of resting spores.

It may therefore be concluded that low temperature is very effective as an additional factor to a certain degree of "overcrowding", i.e. to some changes in the medium which occur in the course of the growth and reproduction of the cells. The importance of the latter factor becomes clear from the following experiment. On 6 November a small number of cells were isolated in a watch-glass and kept at room temperature. During the following days a few auxospores and no resting spores were formed. On the 19th the great majority of diatoms had formed persistent resting spores. On

the 20th the cells were taken out with a fine pipette and counted. The culture contained:

- 6656 resting spores (mostly of narrow cells, some of broad cells developed from auxospores),
- 28 vegetative cells.

In order to find out when persistent resting spores will germinate, and whether they would do so at low temperatures, 1000 resting spores were placed in a watch-glass with fresh culture medium and left at room temperature near the window, and a further 1000 were transferred into a watch-glass with culture medium and placed in an ice-box. The number of germinated spores on the following days is given in Table IV. As soon as any vegetative cells were found—the cultures were examined daily—they were taken out so that no appreciable reproduction of the cells could have occurred. Thus the number of vegetative cells given in the table corresponds closely to the number of spores which had germinated. At room temperature germination started on the 3rd day when 126 vegetative cells were taken out and four auxospores which must have developed from vegetative cells shortly after the germination. During 12 days, counted from the start, altogether 569 resting spores had germinated. After that the culture had to be discarded owing to an infection with bacteria.

TABLE IV—NUMBER OF GERMINATED RESTING SPORES ON THE DAYS FOLLOWING THEIR TRANSFERENCE INTO FRESH CULTURE MEDIUM

	1000 resting spores at room-temperature		1000 resting spores in ice-box (7.8° C.)
	No. of vegetative cells	No. of auxospores	No. of vegetative cells
Nov. 21	—	—	—
22	—	—	—
23	126	4	— (6.1° C.)
24	120	5	—
25	91	1	—
26	44	4	6 (4.4° C.)
27	25	1	—
28	37	3	—
29	45	2	2
30	19	1	—
Dec. 2	40	1	—
	<hr/> 547	<hr/> 22	<hr/> 8

A more unexpected result was produced by the resting spores kept in the ice-box where on the 6th day six and on the 9th day a further two vegetative cells were found. This fact shows that resting spores, provided they are placed in fresh culture medium, are capable of germination at a comparatively low temperature and in almost uninterrupted darkness (they were exposed to light for short periods during the daily examinations). Therefore the quality of the medium seems to be the most important factor for germination. This conclusion is supported by the behaviour of the other 1000 resting spores kept at room temperature where germination took place during

a period when in other cultures with an older culture medium, but not necessarily with a larger number of cells than 1000 per c.c. of fluid, very many resting spores were formed under otherwise similar conditions.

In the preceding experiment the 126 cells found on 23 November were placed in a watch-glass, and next day a further 120 cells derived from spores (Table IV) added. The watch-glass was kept on the same place as the other containing the resting spores. The 246 cells reproduced actively and after 5 days four and later on more resting spores were formed, while in the original watch-glass the germination of resting spores continued. This and other similar observations show that with about 1000 spores in 1 c.c. of fresh medium germination can go on for at least 10 days, whereas the metabolic activity of growing and dividing cells very soon produces changes which result in the formation of resting spores.

There were few exceptions to the rule that in cultures resting spore formation was preceded by a strong increase in the number of cells. In one case forty cells were transferred into a watch-glass. Next day already four resting spores were seen and two cells forming auxospores. After 11 days the culture contained:

59 vegetative cells,
10 resting spores,
6 auxospores.

Thus evidently growth and division of the cells were greatly inhibited. The explanation could be found in the presence of a great amount of bacteria which, after some time, could be seen as a "cloud" at the bottom of the glass dish. Clearly their presence was responsible for what otherwise could only be observed in overcrowded cultures: (1) inhibition of growth and reproduction, (2) formation of resting spores, (3) the persistence of auxospores without any sign of development (see p. 24).

In another case a single cell was isolated in 1 c.c. of culture fluid without washing. Only 4 days later division took place, and when drawn under the microscope many bacteria were seen attached to the diatom cells. After a further 9 days in the original medium three cells were present, all containing an irregular resting spore (fig. 15*a*, Plate 3). Immediately after being placed in fresh culture medium the three resting spores took regular shape (fig. 15*b*) and germinated in the course of several days (fig. 15*c*).

The observations described above were made in autumn and early winter. In January–February the *Ditylum* cultures gave some trouble by producing numerous cells with slightly contracted and highly refractive protoplasts. In spring and summer the formation of resting spores was delayed, less regular, and their number smaller. Subculture 7' contained on 20 March (28 days after the inoculation) not more than a few thousands of resting spores; in later subcultures their number gradually decreased to nought. The same was observed in line C of *Ditylum*, started with a single cell on 29 January, and in another culture from one auxospore. In some overcrowded cultures

many cells were found with a protoplast somewhat contracted and irregular in shape, but the formation of resting spores was extremely rare.

These observations show that the "overcrowding factor" alone is not sufficient to induce resting spore formation, and the question arose whether low temperature applied to dense cultures would result in the production of resting spores.

On 2 May 200 cells of clone C were placed in a watch-glass with 2 c.c. of medium. On the 18th the number of cells had greatly increased, and a number of auxospores and very few resting spores had been formed. The culture was placed in the ice-box ($8.3^{\circ}\text{C}.$) for 3 days, then diluted with culture fluid and the diatoms in a sample of 1 c.c. counted. The estimate gave: 16,400 vegetative cells—mostly of the original narrow type—and auxospores, and 1800 resting spores, i.e. about 10 %. Although this number was far greater than ever observed during spring and summer in cultures kept at room temperature it was very little compared with cultures of a similar degree of density in autumn and winter.

The next question was whether low light intensity had an effect on resting spore formation. Several cultures were placed near a west window facing a wall. No direct sunshine came through and the light intensity was further reduced by a blind. In May the light intensity was—as Mr. H. W. HARVEY kindly found out for me—about 10 % of that near the north window and fell within the range of light intensity during late autumn. Dim light had no effect on dense cultures: the cells stopped division but no resting spores were found. When, however, the culture was first placed in dim light for some time and then exposed to low temperature the effect was remarkable, as the following experiment may illustrate.

On 12 May a rich, not yet overcrowded, culture of *Ditylum* (line C) contained a number of auxospores but no resting spores. At 7 p.m. it was placed in an ice-box ($8.3^{\circ}\text{C}.$). Next morning a small number (200–300) of resting spores were found. In the course of the day no germination took place and the culture was again put into the ice-box. The following day no noticeable increase in the number of resting spores took place. The exposure to low temperature was repeated once more with the same result. On the 16th the culture was placed in a dim light near the west window. After 2 days there was about the same small number of resting spores present—no germination had taken place—and the culture was put into the ice-box ($8^{\circ}\text{C}.$). Next day (19th) the increase in the number of resting spores was quite considerable. The culture was left in the ice-box for another 2 days, then a sample counted. The estimated numbers were:

64,000 vegetative cells and auxospores,
50,000 or almost 45 % resting spores.

This experiment resulted in a number of resting spores nearest to those observed in cultures during autumn. It gives good evidence for the assumption that low light intensity is another factor affecting the formation of resting spores.

When these results were obtained I thought it possible that some irregularities in the number of resting spores formed in the autumn cultures on successive days (see Tables I and II) might be explained by the daily differences in the light intensity, viz. that a small number of spores would only be formed on nights following days with a high quantity of light in spite of the nightly drop of temperature. In Tables I and II data are included giving the vertical illumination integral on the roof of the laboratory in kilolux-hours.* This is a value for the quantity of light during a whole day. It includes, however, direct sunshine, whereas the cultures, placed near the north window, never received direct sunshine but only the amount reflected by a wall opposite the window. At any rate the data do not provide a direct explanation for the irregularities. For instance, on 13 November the amount of light was smaller than on the 15th (104 kilolux-hours), but the number of spores was less on the 14th than on the 16th.

The interaction of light and temperature might, however, be of a much finer and more complicated nature. The minimum of light necessary for resting spore formation probably varies with the temperature, and the temperature effective for the production of many resting spores might vary with the amount of light which the culture had previously received. For instance, on 27 November (Table II) the amount of light might have been sufficiently low to induce the formation of resting spores, but the minimum temperature at night was comparatively very high (11.7°C.), and therefore, perhaps, no resting spores were formed and recorded on the 28th. From the 28th to the 29th, although the minimum temperature was even higher, many spores were formed because the amount of light received on the 28th was very low. This kind of interpretation could be applied to most of the observations summarized in Tables I and II. It remains, however, hypothetical until more data are available, preferably of experiments under different temperatures and light intensities kept constant for long periods.

Apart from the finer mechanism of interaction there seems to be good evidence for the existence of four factors responsible for the formation of resting spores: (1) presence of bacteria—a factor which is probably of little importance in nature; (2) changes of the medium which gradually take place with the growth and reproduction of the cells, possibly consisting of the exhaustion of some nutritive substances; (3) low temperature; (4) small amount of available light. The factor (2) is ineffective alone (summer!), but seems to be the preliminary condition for the action of (3) and (4). Only in pure cultures of a certain density can low temperature and low light intensity induce the formation of resting spores. At a certain density of the culture resting spores were formed temporarily overnight and germinated in the daytime. There was apparently a kind of equilibrium between factor (2) on the one side and factors (3) and (4) on the other. The changes of the culture medium were just so far advanced that under the influence of low temperature resting spores were formed overnight. They have, however, not accumulated to such a degree as to prevent their germination in the

* I am greatly indebted to Dr. W. R. G. ATKINS who kindly provided me with these data.

same medium when the temperature and light intensity increased. With increased density of the culture the effect of factors (3) and (4) result in the appearance of persistent resting spores. These will germinate when transferred into new culture medium under otherwise the same conditions which induce formation of resting spores in dense cultures.

Comparing these results with conditions prevailing in the sea one should consider the fact that the culture medium is much richer in nutrient material than sea water. The population which can be maintained by a unit of "Erdschreiber" is far greater than by the same amount of water in the sea even under optimal conditions. Therefore one would expect a lesser density of the population to be sufficient to induce resting spore formation in the sea than in cultures. For further discussion see p. 41.

(e) *Cell size and auxospore formation*

In *Ditylum*, as in the vast majority of diatoms, the structure of the cell entails a continuous decrease of the cell diameter in a population.* After every cell division the hypotheca of the mother cell becomes the epitheca of one daughter cell. Consequently one daughter cell remains of the same size as the mother cell and the diameter of the other becomes smaller by twice the width of the cell membrane. In some cases (cf. FRITSCH 1935; HUSTEDT 1930) the average cell size of a population was found to decrease progressively according to the binomial law, in others the larger cells seemed to divide more rapidly with the effect that the rate of decrease in size slowed down.

Fig. 27 gives measurements of line A (circles), line C (crosses) and of the Axp.-line and of cells derived from auxospores of line A (triangles). The cells were measured with a micrometer in an eyepiece 12, with an objective 8 mm., and each point represents the average diameter in μ of 50–100 cells.

Line A, started 1 October, had in December an average diameter of 27μ . Within 5 months it decreased gradually to 13.7μ , that is to almost half of the original diameter. After that, these narrow diatoms continued to divide for some days, but no subculture was successful after the end of May. The last measurements of this line were made on 12 May (subculture 11a) and gave an average of 13.7μ , the narrowest cells being 10.7μ in diameter. Subculture 12 was inoculated on 7 May with twenty diatoms. On the 20th this culture was very dense; it contained, however, only broad cells which had developed from auxospores and then reproduced rapidly. Not a single narrow cell of the original size could be found. In other subcultures of 11a the diatoms divided for a few days and then either formed auxospores or perished.

Clone C was started on 29 January, but the culture grew very slowly during February. On 23 April the average diameter was 29.2μ , on 25 August only 11.2μ (fig. 27). Thus the diameter decreased in the course of 4 months by 18μ to about one-third of the original diameter. The rate of decrease was higher in C than in A,

* The only exceptions seem to be *Nitzschia closterium* forma *minutissima* (ALLEN and NELSON 1910) and *Eunotia pectinalis* var. *minor* (GERTLER 1932).

which is probably due in part to a correspondingly higher division rate in the summer as compared with the winter. However, the slope of the curve of C is steeper at the beginning, the rate of decrease greater during the first month than during the later period. Subculture 13 from 14 August was here the last in which the narrow cells still reproduced. On 25 August they had an average diameter of 11.2μ , the narrowest diatoms being 8.5μ . Subculture 14 started on 25 August with seventy narrow cells

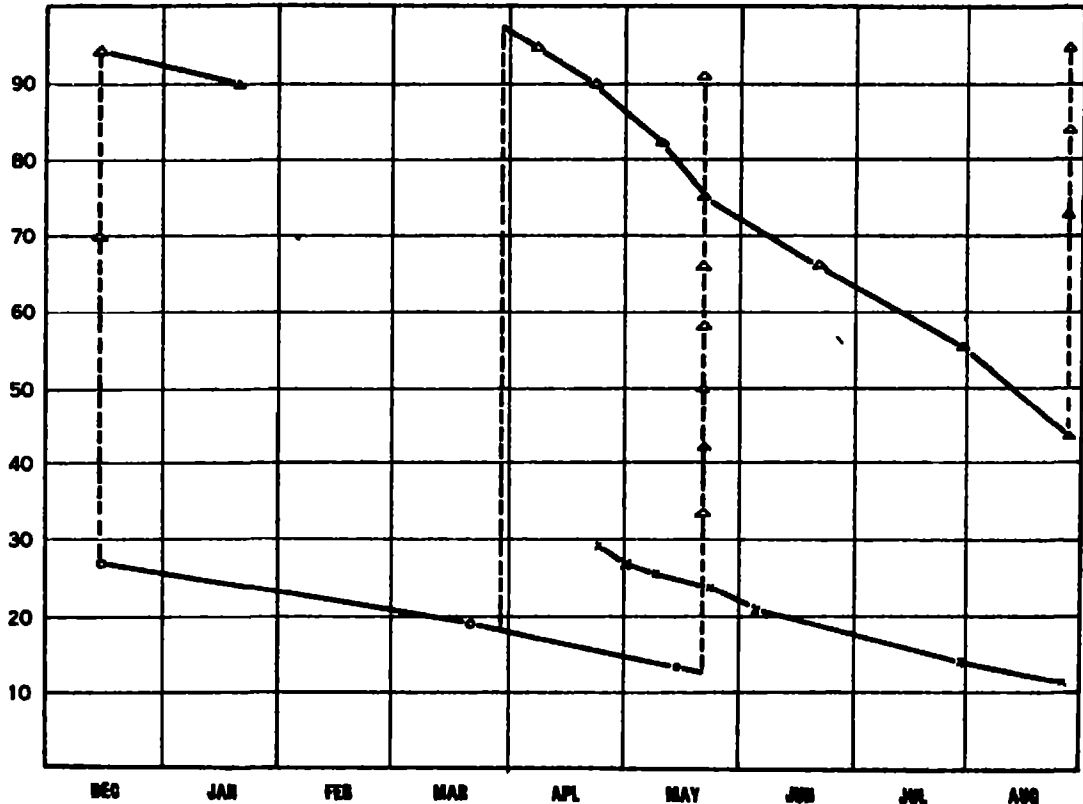


FIG. 27—The average diameter in μ of cells in successive subcultures of *Ditylum*. Line A marked with circles, line C with crosses, the Axp.-line and cells produced by auxospores marked with triangles.

showed 4 days later stages of auxospore formation and broad cells produced by them. A few cells of the original size were present with a very refractive and partly dis-integrated cell content. Thus in *Ditylum* a cell diameter of $8-10\mu$ is the minimum size at which the cells are capable of division.

In both lines formation of auxospores took place at intervals shortly after the cultures had been started until the end, when the cells reached the minimum size and died. The process of auxospore formation is entirely different from that of the resting spore formation. (a) All cells which undergo the formation of auxospores are very long, on the average distinctly longer than the "double cells", i.e. cells which had divided and the two daughter cells not yet separated (fig. 1b, Plate 1). (b) Auxospores are formed outside the cells by the extrusion of the whole protoplasmic content.

(c) The extrusion of the protoplast takes place at the junction of the two halves of the membrane (epitheca and hypotheca). Before the protoplast flows out the cell becomes bent in the middle, the two arms thus forming an obtuse, right or acute angle (figs. 16, 17, Plate 3). (d) The auxospores are more or less spherical bodies, much larger than resting spores, covered with the perizonium, a thin, non-silicified membrane, capable of expansion. They contain a central mass of protoplasm with the nucleus and sometimes with several protoplasmic strands extending towards the periphery. The inside of the membrane is covered with numerous chromatophores (figs. 16*b*, 17*b*, 18*a*, Plate 3).

The increased length of the cells which undergo auxospore formation becomes clear from a comparison between the average length of the "double cells", representing the maximum length during the cycle of growth and division, and the combined length of the two arms of cells in the early stage of auxospore formation (fig. 16*a*, Plate 3). To give an example: subculture 8' (line A) showed on 20 March an average diameter of 19.2μ , and an average length of the double cells of 139.6μ . The average diameter of cells forming auxospores in the same culture was 19.3μ , the combined length of the arms 161.5μ .

This increase in length is most probably not due to actual growth, an elongation of the membrane by the formation of new intercalary bands—although such a process may occur occasionally (see p. 28)—but to the fact that the two halves of the shell separate farther apart than in cell division. As described on p. 4 cell division and formation of the new valves takes place while the parental membrane remains intact, the hypotheca being overlapped by the epitheca. In auxospore formation the growth of the protoplast continues and the thecae are gradually pushed apart.

At the moment of actual separation the bending takes place and the two halves of the shell become set at an angle one to the other. One might assume that the same tension which is present during cell division, and which is there responsible for the contraction of the daughter protoplasts and the formation of the wide gap between them (p. 5), also exists during the growth of the cell preceding auxospore formation. The tension may even become greater, since the growth continues beyond that at cell division. This increased tension would be responsible for the bending at the moment of separation of epi- and hypotheca, and the relief of the tension would be eventually produced by the detachment of the protoplast from the valves and its outflow through the split formed on one side between epi- and hypotheca in the course of bending. In an early stage of this process the outflowing protoplast is often drawn out to a point at the two poles, and as in resting spore formation remains for some time connected by a fine protoplasmic filament with the valves near the insertion of the spine (fig. 16*a*, Plate 3).

If isolated in such a stage the auxospore will be completed within less than 24 hr. Mostly the protoplasm flows out simultaneously from both arms (fig. 16*a*, Plate 3) and the empty shells may remain for some time loosely attached to the auxospore. (They were, in fact, in contact with the auxospore represented in fig. 16*b* (Plate 3))

but became detached when pipetted on a cavity slide for drawing.) Sometimes, however, the protoplasm leaves one arm first while still occupying part of the other (fig. 17*a*, Plate 3). Since the empty shell drops off very easily the remaining arm with the outflowing protoplasm might suggest a formation of auxospores different from that described above and similar to that of *Melosira Borreri* (p. 35). However, in all more closely observed instances of auxospore formation in *Ditylum* it was preceded by the bending of the cell.* Thus the cell drawn in fig. 17 (Plate 3) was bent at an angle of about 120° when first noticed (11 a.m.); the outflow of the protoplasm had not yet started. At 2.30 p.m. the stage drawn in fig. 17*a* (Plate 3) was reached. The empty arm was in close contact with the young auxospore when examined first; during the transport to the microscope it had been moved away a bit. Next day, perhaps already during the night, the auxospore was completed (fig. 17*b*, Plate 3). The whole process of auxospore formation does not take more than 24 hr.

Newly formed auxospores either develop immediately into broad cells in the culture medium in which they have been formed or, in old cultures, persist as spherical bodies and develop only after being placed into fresh culture medium (see p. 24). A few stages of the development of a single, isolated auxospore are drawn on fig. 18 (Plate 3). The day after having been placed into fresh culture medium the auxospore had flattened on one side and formed a valve and a spine. (The shrinkage of the protoplast from the perizonium only occurred when transferred on a cavity slide.) Next day the opposite side was flat too and another valve and spine had been formed. The cell had, however, not yet taken its ultimate prism shape, with triangular valves (fig. 18*d*, Plate 3). This final stage was reached after one or two divisions and remained a characteristic of the whole progeny of the cell for several months. Afterwards the shape became more and more cylindrical. The perizonium was thrown off during the formation of the valves (fig. 18*e*, Plate 3). The cell produced by an auxospore is always considerably broader in diameter than the cell which produced the auxospore, and given the right conditions divides immediately after formation.

What are the conditions responsible for the formation of auxospores? It was mentioned above that both lines A and C formed auxospores shortly after the start of the cultures. This statement should be understood in the sense that the cells were capable of forming them under certain conditions. If, however, a culture is made from a big cell picked out from a plankton sample, or derived from a big auxospore, the resulting population is not capable of forming auxospores for a considerable length of time. This fact also applies for the pennate diatoms (GEITLER 1932).

In fig. 27 measurements are given of a line (Axp.) derived from an auxospore at the end of March. On 7 April the average diameter was 94.2 μ . The rate of decrease in the following months was much greater, the slope of the curve being much steeper than in lines A and C. In 5 months the diameter decreased by more than half to 43.7 μ

* A similar stage of auxospore formation as on fig. 18*a* (Plate 3), with the protoplasm extruded half way out of one arm, has been found by KARSTEN (1899) in the plankton.

(26 August). Although the division rate was not studied in detail occasional countings left little doubt that it was equal to or rather smaller in the Axp.-line than in line C. The division rate of the latter only slowed down in July/August when the cells approached their minimum size and showed a general decrease of vitality. But up to the last subculture the cell diameter decreased and auxospores were formed. In this respect they differ from the "under normal sized" cells in cultures of pennate diatoms (GEITLER 1932) which may be cultured for some time but have lost the capability of forming auxospores.

Since the external conditions were the same for the Axp.-line as for line C the more rapid decrease of the diameter can only be explained by the assumption that in cells of big diameter the cell wall is thicker than in narrow ones, and that with the gradual decrease of diameter a proportional decrease of the thickness of the cell wall takes place with the effect that the rate of decrease of the diameter is slowed down. This is again in agreement with GEITLER's observations on pennate diatoms.

The first auxospores of the Axp.-line were found on 25 August in the dense subculture 13 with cells of an average diameter of 43.7μ . They were few in number, and there were also two small colonies of broad cells which originated from auxospores with a diameter varying from 72.6 to 94.0μ with an average of 83μ (fig. 27). We may conclude that a cell diameter of about 43μ is a preliminary condition for auxospore formation. Lines A and C showed that cells with a diameter less than 43μ are capable of forming auxospores but need not do so until they reach a diameter of about 10μ , when further reproduction seems to be impossible for some physiological reason and they either form auxospores or perish.

The diameter of 43μ can be regarded as an internal factor which must be realized before auxospore formation can take place. There follows a period of 6 months—under comparatively very favourable conditions in the laboratory—during which environmental factors are responsible for the formation of auxospores. This conclusion may be drawn from the following observations.

If auxospore formation were only due to internal factors one would expect this process to take place independently of the age of the cultures. This, however, was not so. Table V gives the dates when the first auxospores or cells forming auxospores appeared in successive subcultures of lines A and C. In the first subcultures of both lines auxospore formation only occurred in old, very dense cultures, considerably later than the formation of resting spores (see Tables I–III). When transferred into fresh culture medium the cells rapidly divided, and again only after 16–30 days when the culture had become crowded did auxospore formation take place. The same could be found in later subcultures, in line A for 5 months, in line C for about 2 months. After that, when the populations have reached an average diameter of about 20μ , the intervals between the start of the culture and the appearance of the first auxospores became continuously shorter, being finally only 2–3 days (Table V). In some of the final cultures auxospores were even found 1 day after the inoculation.

TABLE V—RELATION BETWEEN AGE OF THE CULTURE
AND FORMATION OF AUXOSPORES

No. of subculture	Culture started on	First appearance of auxospores after days
	Line A	
1	Oct. 5	18
2	Oct. 21	16
3	Nov. 1	19
4	Nov. 11	22
5	Nov. 28	29
6	Dec. 16	28
7	Dec. 27	17
7'	Feb. 20	14
8'	Feb. 26	9
9	Mar. 6	5
10	Mar. 24	7
10a	Mar. 31	6
11a ₁	May 12	3
	Line C	
2	Mar. 4	20
3	Mar. 12	19
4	Mar. 31	27
4A	April 24	8
8	June 4	7
8''	June 26	6
13	Aug. 25	2

Thus the formation of auxospores, like that of resting spores, seems to depend on certain changes of the medium occurring with the growth and reproduction of the diatoms in a culture. The degree of the changes necessary for the formation of auxospores becomes smaller with the decrease of the cell diameter of a population or, in other words, the smaller the diameter the more ready the cell to respond to those changes and to undergo transformation into an auxospore.

Another phenomenon is connected with the different intervals at which auxospores were formed in successive subcultures. In the first culture of both lines A and C the number of auxospores produced was very great; there were thousands of them when the cultures became dense. There was, however, only a negligible number of broad cells developed from them. The auxospores persisted throughout two to three weeks, or even longer, until the culture decayed and only developed into broad cells when placed into fresh culture medium. Whereas newly formed auxospores developed regularly within 2 days, older ones needed up to 5 days for the formation of broad cells. When in later subcultures the auxospores were formed in younger cultures they all developed into cells of bigger diameter and both types of cells divided actively until the cultures became very dense. In the subcultures of line A made in April/May and in those of line C made in July/August the majority of cells present after several days were those of the broad type and their preponderance increased with the age of the culture. Finally, it became difficult to find and pick out even a few of the narrow cells for

subculturing and, as stated above, those transferred either formed auxospores or soon perished.

With regard to the nature of the changes in the medium which appeared to be responsible for the formation of auxospores some experiments with pure sea water as medium may be mentioned. On 4 June sixty cells were taken out of subculture 7 of line C, washed five times in sterile sea water, then thirty cells placed into a Petri dish containing sterile sea water only (*a*), and thirty cells into another Petri dish containing the usual culture medium (*b*). Next day one cell forming an auxospore was seen in (*a*), none in (*b*). On the 6th eight auxospores and two knee-shaped cells were found in (*a*), none in (*b*). On 11 June, after a week, the cells of both cultures were counted. The sea water culture (*a*) contained:

35 vegetative cells
21 auxospores
<hr/> 56

The "Erdschreiber" culture contained:

378 vegetative cells
5 cells forming auxospores
0 auxospores
<hr/> 383

As another control the "Erdschreiber" culture 7*a* may be mentioned which was started on 5 June with fifteen cells and contained after a week:

638 vegetative cells
9 cells forming auxospores
0 auxospores
<hr/> 647

This experiment shows that diatoms with a diameter of about 20μ (see fig. 27) not only divide very slowly in sea water as compared with "Erdschreiber" but that auxospore formation begins sooner, within a day in sea water, after 7 days in "Erdschreiber". The degree of "overcrowding" necessary to induce auxospore formation is obviously much smaller in sea water. This may, perhaps, be explained by the fact that "Erdschreiber" contains a much greater amount of nutrient substances than sea water. One might compare sea water with uncultivated soil and "Erdschreiber" with manured garden soil.

On 23 May 100 cells of line C were placed in sterile sea water (*a*), twenty cells from the same culture in "Erdschreiber" (*b*). On 27 May not a single auxospore

could be found in (*b*) whereas the majority of (*a*) consisted of auxospores. On 4 June the content of (*a*) was counted. There were:

15 vegetative cells
557 auxospores
3 resting spores
<hr/>
575

Thus only few divisions had taken place and almost all cells had undergone auxospore formation. Culture (*b*) had to be used for another purpose and could not be counted. It was by then a rich culture with many thousands of cells, at most 5 % auxospores and some broad cells produced by auxospores.

Of the 557 auxospores in the sea-water culture (*a*) not a single one had developed or showed signs of development into broad cells. On 4 June thirty auxospores were transferred into a watch-glass with sterile sea water, thirty into a watch-glass with "Erdschreiber". No development took place in sea water; in "Erdschreiber" four auxospores had formed one valve with spine on 6 June and by the 10th twenty-four cells of big diameter had developed.

On 10 June twenty auxospores were transferred from the original sea water culture into "Erdschreiber". After 2 days one broad cell was found, and a further six were formed by the 17th. The rest had shrunk in the meantime and were not capable of development. The auxospores which had remained in the Petri dish with sea water did not show any sign of development until the beginning of July and then shrunk completely.

All these observations seem to show that the progressive changes of the culture medium which induce auxospore formation are produced by gradual exhaustion of substances which are present in "Erdschreiber" in larger quantities than in sea water, rather than by an accumulation of some metabolic products. Sea water seems to have a similar effect as the "Erdschreiber" of an overcrowded culture, where cells are induced to form auxospores which are, however, not capable, or capable only to a very limited extent, of developing into vegetative cells unless transferred into fresh culture medium.

Apart from the cell size and the changes taking place in the culture medium no other factors seemed to have any effect on either the time of appearance or the number of auxospores. They were formed under conditions described above throughout the year, whereas, as we have seen, resting spores were not formed in summer.

So far only the cell diameter has been considered. It has been shown that with the growth of a population the average diameter gradually decreased and that a regulation may take place when the diameter reached a certain size by the formation of auxospores and their development into cells of much bigger diameter. In this respect *Ditylum* shows very similar conditions to the pennate diatoms. There is, however, one

point in which they differ. GEITLER (1932) found in the Pennatae that the size of auxospores and of the cells produced by them is practically constant for every species. SCHREIBER (1931) observed in a centric diatom, *Melosira nummuloides*, that the size of auxospores is unusually small when formed in a medium of high salt concentration. These small auxospores, however, did not develop but burst the membrane on the following day, when the protoplast was extruded and a new auxospore of normal size formed.

In *Ditylum* the size of the auxospores varies quite considerably, and the range of variation increases with the decrease of the cell diameter of a population. Fig. 27 shows that in line A the broad cells produced by auxospores in December varied from

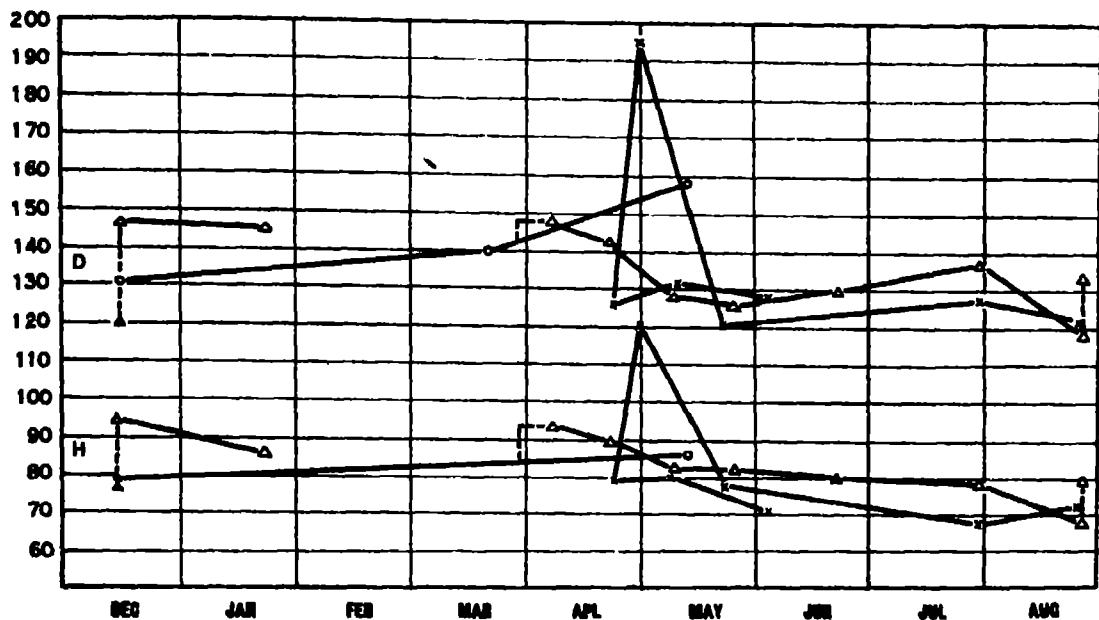


FIG. 28—The average length in μ of cells in successive subcultures of *Ditylum*. Marked as in fig. 27.

70 to 94μ in diameter. Similarly the cells developed from auxospores in the Axp.-line in August varied in diameter from 73 to 95μ with an average of 84μ . At the end of May, when the cells of line A reached the diameter of 13μ , the cells developed from auxospores varied from 33 to 90μ . Thus cells were produced by auxospores which in view of their diameter would be expected to form auxospores as soon as overcrowding of the culture occurs. This was actually so. On 5 June in subculture 12—the same from which a sample had been measured on 20 May—a number of cells with a diameter from 30 to 40μ were found to have formed or be forming auxospores; cells which had themselves developed from auxospores only 2–3 weeks ago. The broad cells produced by auxospores in line C have not been measured but the general behaviour was very similar.

In pennate diatoms the pervalvar axis—corresponding to the long axis of *Ditylum*—remains practically constant (GEITLER 1932). In *Ditylum* there exists on the other hand

a considerable variation which shows no correlation with the decrease of the diameter and its increase after auxospore formation. Since the individual growth of the cells takes place in a longitudinal direction not all cells are suitable for measurement and comparison. Two stages appear obviously to be the best, viz. the "doubles", cells which have undergone division, the daughter cells not yet being separated (fig. 1 *b, d*, Plate 1), and the "half-cells", daughter cells shortly after their separation with the new valve still lying within the epitheca (fig. 1 *c*, Plate 1). The latter measurement is evidently the more reliable one, since the length of the "double cells" may vary according to the degree of separation of the daughter cells. The difference between the length of "double cells" and "half-cells"—the distance between the two curves on fig. 28—represents the range of individual cell growth. The average length in these two stages of line A, C, and Axp. are plotted on fig. 28. The dates of the measurements are the same as for the diameters on fig. 27.

In line A the "double cells" (= D) had an average length of 131μ , the "half-cells" (= H) of 79μ in December. In March the D cells were 139.6μ ; of the H cells only three were measured, they were 65, 75, and 80μ respectively long. In May the D cells showed a marked increase to 157.8μ , the H cells to 85.4μ .*

That this increase in length is not necessarily connected with the decrease of the diameter becomes clear from the measurements of cells of other lines. In clone C (fig. 28) the average length of D cells was on 23 April 125.8μ , of H cells 79μ . On the 24th two subcultures were made: subculture 5 with forty cells in a Petri dish, subculture 4A with fifty cells in a watch-glass. On 1 May the number of cells in 4A was greatly increased, but the cells were unusually long. 100 cells were measured; the average length of D cells was 194.8μ , that of the H cells 120.5μ . A sample of 100 cells of subculture 5 was measured on 8 May. It showed only a slight increase in length as compared with that in April: the D cells were 131.2μ long, the H cells 80μ . On 2 May twenty-five of those extremely long cells of subculture 4A were washed and placed in a Petri dish. On the 22nd a sample of 100 cells from this culture gave an average cell length of 120μ for the D cells, of 77.1μ for the H cells. The length of the cells had become "normal" again.

Unfortunately it was impossible to discover the cause of the enormous growth in length of the *Ditylum* in subculture 4A. It might have been an infection of the watch-glass culture with some bacteria which had no effect on the division rate but had an effect on cell growth.

In later subcultures of line C the length of the cells did not differ much (fig. 28). The values obtained in August compared with those of April show on the whole a slight decrease in length.

* In all lines the D cells appeared to be considerably smaller than the double length of H cells. This is due to the fact that only those D cells could stand the pipetting and transferring on glass slides (for measurement) which had the original hypotheca well overlapped by the epitheca. D cells with the daughter cells being far advanced in separation broke apart during that procedure and arrived as two H cells on the glass slide.

There is also no definite correlation between the length of narrow cells and that of broad cells derived from auxospores. Among the cells developed from auxospores of line A there were from the beginning some longer and some shorter than the cells by which the auxospores had been formed (fig. 28, December). The Axp.-line showed in April an average length of cells of 147μ (D cells) and 93.5μ (H cells) respectively, slightly higher values than those of line A at the same time (fig. 28); in August it was distinctly smaller than in April.

All these facts show that there is no correlation between changes in the length and the progressive decrease of the breadth of *Ditylum*. Whereas the decrease of the diameter is determined by the structure and division process peculiar to the diatoms there apparently exist environmental factors affecting the extent of the individual growth in length of cells. From the slopes of the curves of fig. 28 it may be justifiable to assume that under more constant culture conditions—i.e. controlled temperature and light, and more frequent subculturing—the variability of the length of the cells would be greatly reduced and the length would remain practically constant in successive subcultures.

In spite of the variation of the length of cells in the cultures there is no doubt that on the whole the cell volume decreased with the decrease in diameter.

No special study was made of the ratio of length to breadth of cells developed from different auxospores, but it was found to be extremely variable even within the same culture.

It was mentioned in the introduction that until quite recently the formation of auxospores in centric diatoms had been regarded as a growth phenomenon not preceded by any nuclear changes or sexual phenomena. Both reduction division and the sexual phase were rather vaguely connected with the formation of microspores. Only PERSIDSKY (1929, 1935) described the occurrence of reduction division and autogamic sexual fusion of two out of four nuclei previous to auxospore formation in two *Chaetoceros* species and in *Melosira varians*. Particularly for the latter species the description and drawings of some important stages of the reduction division and nuclear fusion are rather convincing. v. CHOLNOKY (1933) found young auxospores of *Melosira arenaria* containing one big and two small nuclei, which he interpreted as being the result of a reduction division and the formation of four nuclei, of which two fused while the remaining two degenerated.

Cytological observations on *Ditylum* give a similar support to PERSIDSKY's views. From the outset it was found that in every culture in which formation of auxospores and their development had taken place the broad cells contained a much bigger nucleus than the original narrow ones (fig. 19a, b, Plate 3). This alone would be difficult to explain on the assumption of growth only. Nuclei which were about twice as big as those of the sister cells were also found in bent cells before the actual formation of auxospores. Cells whose protoplast had been partly extruded in the course of auxospore formation frequently contained two nuclei (fig. 19c, Plate 3), mostly without

nucleoli, or with one nucleus again much larger than any nucleus of vegetative cells. One cell, shortly before the formation of the auxospores proper, contained one big nucleus with rather indistinct boundaries (shortly after fusion?) and two small degenerating nuclei (fig. 19*d*, Plate 3). No nucleolus could be seen in the big nucleus. The most instructive stages so far found were several young auxospores containing one big nucleus lying near the periphery of the auxospore within a protoplasmic area and two small nuclei similarly situated on the opposite side of the cell (fig. 19*e*, Plate 3).

As yet no stages preceding the appearance of two or three nuclei as described above have been found. But even these incomplete results, similar to those of v. CHOLNOKY, taken in conjunction with the more complete results of PERSIDSKY, leave little doubt that the auxospore formation in *Ditylum* is, or may be, preceded by a reduction division, the first meiotic division resulting in the formation of two nuclei, the second in four, two of which fuse while the others, taking no part in this very reduced sexual process, degenerate.

No microspores were found in *Ditylum*, and although that would not be sufficient proof for their non-existence the conclusion may be safely drawn that they do not take part in the formation of auxospores. The cultures of *Ditylum* were examined at frequent intervals for about a year under the binocular microscope, and many samples, both living and preserved, were studied under high power. No structures or bodies, whether flagellate-like or not, were observed apart from vegetative cells, resting spores, auxospores and occasionally bacteria. Thus the possibility of a fertilization of cells by microgametes before the formation of auxospores, suggested by WENT and GEITLER (see p. 2), certainly does not apply to *Ditylum*.

CHAETOCEROS DIDYMUS EHR.

Ten chains of this species were isolated from a plankton sample of 18 September 1935. On 21 November the culture was continued as a clone. This diatom forms very long chains which remain suspended until the culture becomes very dense. Then the majority of chains are found lying at the bottom of the glass dish. It may be noted that the long chains are slightly bent, not straight as usually described (GRAN 1908). The reason is probably that in tow-nettings long chains are broken into several pieces which then appear straight. Long chains of broad cells are also always slightly twisted, showing the flat surface in front, the narrow side view in the middle and again the broad surface at the end of the chain.

Two varieties are distinguished (MEUNIER 1913; GRAN 1908; LEBOUR 1930; HUSTEDT 1930): v. *genuina* with rather strong bristles crossing one another near the base, and v. *anglica* with fine bristles crossing far outside the chain. Both types occur in clone cultures at various periods (figs. 20–22, Plate 4), all representing specimens from one clone). It seems that chains of narrow cells which are capable of auxospore formation mostly belong to the type of v. *genuina* (fig. 22, Plate 4), whereas in broad chains

the bristles may cross either near the base (fig. 21, Plate 4) or farther off (fig. 20*b*, Plate 4).

Resting spores have been described by MEUNIER (1913). In the cultures they were mostly found in spring, lying singly within normal, not specially formed mother cells (fig. 21, Plate 4). Apart from the fact that resting spores of *Chaetoceros didymus* were also found in dense cultures only, the environmental conditions for their formation must be somewhat different from those in *Ditylum*. They were never found in such great numbers as in *Ditylum*—this applies also to all other diatoms which I cultured—and were not regularly formed in successive subcultures throughout a long period.

The first auxospores were noticed on 29 October in a 25 days old culture of the line mentioned above. These auxospores did not develop in the original culture. The first chains of broad cells were found in a later subculture on 11 November. They appeared in all subsequent cultures, always at a considerable interval—8–28 days—after the start of the culture.

The auxospores are similar to those described in other species of *Chaetoceros* (cf. FRITSCH 1935). The mother cell remains straight and the auxospore is formed laterally, the protoplast being extruded through a split between the epitheca and hypotheca of the cell (fig. 22, Plate 4). The auxospores develop immediately after their formation or, in very dense cultures, persist until they perish. On 18 March a chain containing an auxospore was isolated, on the 19th the auxospore had given rise to a broad cell which had divided in the meantime (fig. 22*b*, Plate 4). Behind the cells the empty perizonium was attached to the mother cell and in a neighbouring cell auxospore formation had started. On the 20th there were three broad cells, but the young auxospore was not yet completed. A small colony of bacteria was seen attached to it which evidently caused the delay. The other cells of the same chain continued to divide. On the 19th three cells were in front of the auxospore, next day six. The cells behind had divided into four, three of which broke off when the chain was transferred on a cavity slide. The broad cells developed from the auxospore produced a chain at right angles to the long axis of the parental chain and broke off on the 22nd.

As can be seen from fig. 22 (Plate 4), the empty mother cells of the auxospores are distinctly longer than the vegetative cells. On 23 March the average length of twenty-five "double cells" was 36.8μ , that of twenty-five auxospore producing cells from the same culture 46.4μ . If cells which undergo auxospore formation do not grow longer than the vegetative cells we must assume that in the latter the epitheca overlaps the hypotheca along a distance of 5μ .

Measurements of the cell diameter are given in fig. 29. In March the average diameter of the original line (from September) was 7.5μ . It had decreased to 3.8μ by June, after which no further subculture was successful. The cells which did not form auxospores were no longer capable of division and died. Broad cells developed from auxospores in March had a diameter of 17.3μ . Those of a culture started with six broad chains on 20 January showed a diameter of 22.1μ in April (fig. 29, Axp.).

There followed a rapid decrease to 7.6μ in September, that is a decrease by about two-thirds of the original diameter during 5 months. As in *Ditylum* the rate of decrease is much greater in broad cells than in narrow ones.

The Axp.-line of broad cells did not produce auxospores until the middle of August. The first auxospores were produced by cells with a diameter of about 9μ . This diameter, with a certain range of variation, must therefore be regarded as the maximum size of cells capable of forming auxospores. A population of *Ch. didymus* with a diameter

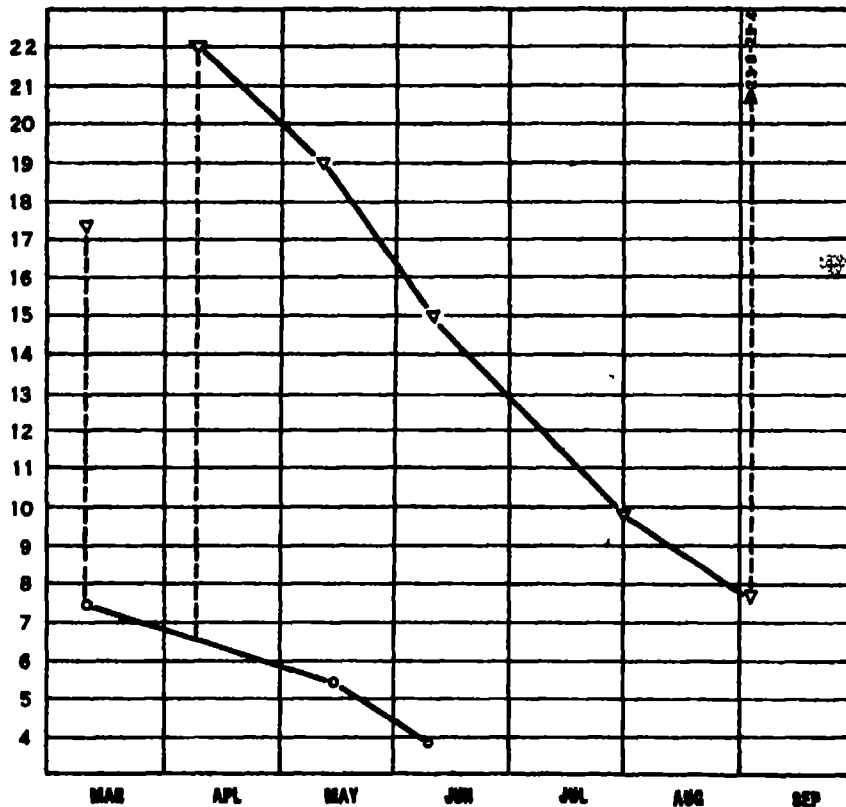


FIG. 29—The average diameter in μ of cells in successive subcultures of *Chaetoceros didymus*. The original narrow line marked with circles, the Axp.-line and cells developed from auxospores marked with triangles.

of 30μ —the size of the original cells of the Axp.-line which we may estimate from the slope of the curve—must therefore reproduce for a period of 7 months under very favourable conditions before the cells obtain an average diameter of 9μ . Then a period of 3 months follows when, as in *Ditylum*, environmental factors may induce some cells to form auxospores. That the main factor again changes in the medium produced by the rich growth of a culture may be concluded from the fact that whenever a new subculture was made a period of reproduction by division followed varying from 12–28 days in relatively broad cells to 1–2 days in narrow cells approaching a size at which division stopped and the cells died.

By transferring samples at frequent intervals (4–7 days) in fresh culture fluid it was possible to eliminate the formation of auxospores in two lines for a period of 6 and 8 weeks respectively. The smaller the diameter of the cells the more difficult it becomes to eliminate auxospore formation.

With regard to the size of auxospores and the diameter of cells produced by them a certain variability can be seen in the values given in fig. 29 for the broad cells in March and those formed in April. It must, however, be pointed out that as in *Ditylum* the variability greatly increases with the decrease in diameter of the cells producing the auxospores. In the subculture from June containing cells of the original line with an average diameter of only 3.8μ the majority of cells produced by auxospores were of a diameter varying from about 7 to 12μ , a size at which the cells become capable of forming auxospores again. Under low power it becomes difficult to distinguish these cells from those of the original line and to pick out the narrow chains for subcultures without contamination by cells developed from auxospores.

When in the Axp.-line the cells reached the diameter of about 9μ the first auxospores formed in overcrowded cultures in August and September did not develop in the original medium. It was only after having been transferred into fresh culture medium (1 September) that the auxospores produced broad cells and chains after 5–7 days.

The length of the cells shows no regular increase or decrease in successive subcultures but fluctuates in both narrow and broad cells between 32 and 41μ ("double cells").

CHAETOCEROS PSEUDOCRINITUS OSTENF.

The culture was started with one chain isolated from a plankton sample on 31 January 1936. In March the average diameter of the cells was 15.1μ ; it decreased to 7.2μ in August. The first auxospores were formed in October. Occasionally other cytological phenomena were observed which shall be briefly described.

In very overcrowded cultures, or in dense cultures contaminated with bacteria, where reproduction had practically ceased and many chains showed signs of disintegration, cells were found containing mostly two to four (fig. 23, Plate 4), sometimes six ellipsoid-shaped bodies instead of the normal protoplast. When there were three such bodies (fig. 23a, b, Plate 4), one was much bigger than the rest, apparently lagging behind with division. Most of them contained a nucleus near the centre and distinct chromatophores at the surface; some were more or less empty and obviously dead (fig. 23c, Plate 4, lower cells).

The interest of these structures lies in their resemblance to what has been described by various authors as reproductive bodies or, later, microspores (see p. 38). It may therefore be pointed out that there is no evidence whatever that these microspore-like bodies found in the cultures of *Ch. pseudocrinitus* acted as reproductive cells. They were never liberated or at any rate never found outside the parental cells, and flagellate-like cells were never found in the cultures where their formation had taken place.

Chains with cells containing such bodies were isolated into watch-glasses with fresh culture medium. There the neighbouring cells often recovered and started dividing again, but no trace of microspores in the shape of motile or non-motile gametes or asexual swimmers could be found.

Under similar conditions the formation of such bodies also occurred in *Ch. didymus* cultures but less frequently.

As they only occurred in very overcrowded cultures, or in such which were strongly contaminated with bacteria, their formation might perhaps be explained by the assumption that under certain unfavourable conditions cell division is not followed by the separation of the thecae and the formation of new valves. The nucleus retains the capability for further division which is followed by the division of the protoplasm and the formation of "daughter cells" inside the old membrane. It would thus be a degenerative process similar to the so-called plasmogamies in *Thecamoebae*. In these protozoa BELAR (1921) found that in every dense agar-agar culture abnormal cells appeared after some time: viz. big cells with two or more nuclei the division of which has not been followed by cell division, or several cells which, derived from a single cell by division, were unable to separate and remained fused at the base of their pseudopods.

SKELETONEMA COSTATUM (GREVILLE)

This diatom divides at a rate of one to two divisions per day. It forms long, mostly straight chains, but in every culture a considerable number of curved and spiral-shaped chains can be found. The spaces between the cells are usually rather short (fig. 24*a, b*, Plate 4). This mode of growth is in agreement with KARSTEN's observations (1898) that the spaces are short in still water, and long in cultures which had been shaken.

The culture was started with six chains from a tow-netting on 23 September 1935 and continued as a clone on 19 November. The first broad chains developed from auxospores were found on 27 March (fig. 24*a, b*, Plate 4). It seems probable, however, that auxospores were formed some time before and were overlooked, since also in later subcultures they showed far less readiness for development than *Ditylum* and *Chaetoceros* and remained in great numbers at the bottom of the Petri dishes. Even when transferred into fresh culture medium only about 50% will develop after 5-8 days (compared with 1 and 2 days for *Chaetoceros didymus* and *Ditylum* respectively). Also the number of auxospores found in crowded cultures was small compared with *Ditylum*. They were formed by cells bent to \pm right angles (fig. 24*c*, Plate 4) and were never found to develop in connexion with the parental chains as in *Chaetoceros*.

In April the original line showed a cell diameter of 3μ ; broad cells that of 11.3μ (fig. 30). The diameter of the narrow cells decreased to 2μ in June, and it became difficult to distinguish them from chains of an average diameter of only 5.4μ developed from auxospores. A line (Axp.) started from a single broad chain in March possessed

a diameter of 11.4μ in April. The next measurement in May gave only the slightly smaller value of 11.3μ . Either the sample of sixty chains measured was too small or the division rate was lower than usual. In September the diameter had decreased to 8.5μ .

Resting spores (fig. 24*d*, Plate 4) were observed only once in May when a small number was formed after the exposure of a dense culture to dim light for 4 days and subsequently to low temperature (8.3°C.) for 3 days. Low temperature alone seemed to have no effect.

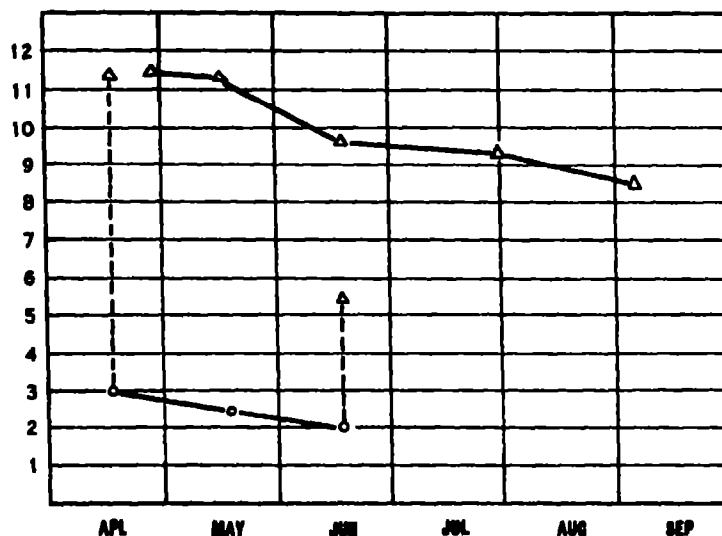


FIG. 30—The average diameter in μ of cells in successive subcultures of *Skeletonema costatum*. The original line marked with circles, the Axp.-line and cells produced by auxospores marked with triangles.

MELOSIRA BORRERI GREVILLE

This is a coastal bottom form with detached strands occurring occasionally in the plankton. In culture the end-cells of the filaments attach themselves to the bottom of the glass dish. Auxospores have been described by MIQUEL (1892) and KARSTEN (1899). Usually they appear as end-cells of narrow filaments, one theca of the mother cell and the rest of the chain becoming disconnected in the course of their formation. Occasionally, however, they remain connected with the neighbouring cells at least until the auxospore has formed a new epitheca (fig. 25, Plate 4).

The culture was started with one narrow chain on 1 April. On the 18th a few auxospores were found and two short chains of broad cells. The latter were isolated and cultured separately (Axp.-line). On 13 May the narrow cells had a diameter of 10.9μ , and the cells of the Axp.-line one of 18.6μ (fig. 31). Later, the narrow cells produced an ever-increasing number of auxospores, and in June broad chains had completely overgrown the original line. The Axp.-line produced no auxospores until 5 July. In August many auxospores and broad chains were formed with an average

diameter of 18.6μ , that of the original Axp.-line having decreased in 3 months by 8.5 to 10.1μ .

SCHREIBER (1931) has shown that *M. nummuloides* can stand salinities varying from 0.5 to 6% . If cells of a diameter of 12μ , capable of auxospore formation, were transferred into a medium of higher concentration, no auxospores were formed. When placed into lower concentration they were formed readily, the relative not the absolute salt concentration being the decisive factor.

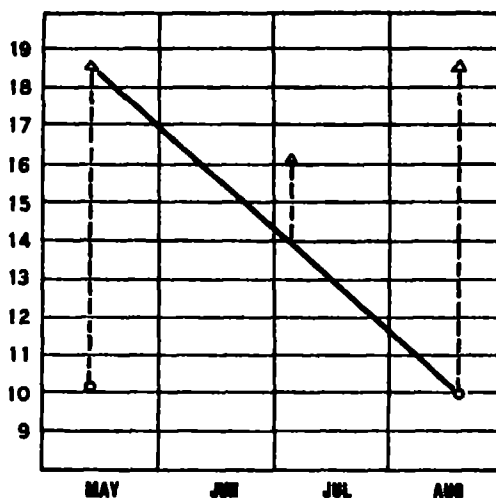


FIG. 31—Showing the differences in the size of the diameter of narrow cells (circles) and cells developed from auxospores (triangles) in *Melosira Borreri*.

NOTES ON OTHER SPECIES

In December 1935 the plankton contained a small number of very broad *Coscinodiscus* cells. One cell was picked out on 18 December; the cells of the resulting culture had an average diameter of 188.2μ with a maximum of 195μ on 22 January. It decreased to 139.7μ by the end of July 1936. No auxospores have been yet formed (November 1936). The species was difficult to identify. According to Miss LEBOUR, the type to which it showed the greatest resemblance was *C. obscurus*. This pelagic species, however, has so far only been found in Languedoc and in the North Atlantic (HUSTEDT 1930, p. 419).

In January two cells in a sample of the first culture showed a curious phenomenon. The girdle, or perhaps the connecting band of one theca only, had burst, the two ends being detached from the cell (fig. 26, Plate 4). In subculture 1 six cells out of a sample of forty-three showed the same phenomenon. In some only a short piece of the connecting band was attached to the cell, the rest having apparently been thrown off. In February they numbered twenty-six in a sample of 400 cells; in April only two were found among 100 cells, and in subsequent cultures they did not occur any more.

At first I was tempted to regard this process as a regulation of the cell diameter independent of auxospores.* The rigid girdle being broken the cell could expand and form a new and broader girdle and valve. But, as mentioned before, on the whole the diameter of the cells of successive cultures showed a steady decrease. Thus, should that phenomenon in fact represent a secondary growth, it might have slowed down the rate of decrease of the size in the population but did not prevent it altogether.

C. radiatus EHR. has been cultured since 23 September 1935, when two cells were isolated from a plankton sample. In February the average diameter of the cells was 110μ , in August 69μ . No auxospores have been formed yet (November 1936).

Two lines of *C. Granii* GOUGH have been kept in pure culture, one from 31 October 1935, the other from 11 March 1936. The diameter of the cells showed a steady decrease, but in neither of the lines has auxospore formation been yet observed.

GENERAL CONSIDERATIONS

The observations on cultures described above showed that in all the species investigated, even in a thin-walled form like *Ditylum Brightwelli*, the average cell diameter of a population is bound to decrease progressively. The life cycle of centric diatoms appears to consist of successive periods of reproduction by cell division and of formation of auxospores. The diameter of cells developed from auxospores is twice to ten times larger than that of the mother cells. The process of auxospore formation only occurs when the cells have diminished to a certain size. When the cells have reached this size they become capable of producing auxospores but need not do so. They continue to divide, and for a considerable length of time environmental factors decide whether and to what extent auxospores shall be formed. The cell diameter continues to decrease until it reaches a minimum size and the cells either form auxospores or die.

In this respect the centric diatoms differ from the Pennatae (see p. 23). Another difference is found in the fact that in centric diatoms the auxospores formed by cells of different diameter show a great variability in size, whereas this is rather uniform in pennate diatoms.

Cytological studies in *Ditylum* have produced some evidence in support of PERSIDSKY's views, that a reduction division and an autogamic sexual process precedes the formation of auxospores in centric diatoms. The observation of young auxospores of *Ditylum* containing one big and two small nuclei especially strengthens the probability of the occurrence of these processes in *Ditylum*. The possible interpretation of the stages of reduction division described by PERSIDSKY as preceding the formation of macrogametes which need to be fertilized by microgametes or microspores (GETTLER 1932), was eliminated by observations on cultures of *Ditylum*, *Chaetoceros*, *Skeletonema* and *Melosira*.

* The "dogma" of the auxospores being the only means for size regulation has been repeatedly attacked (GEMEINHARDT 1927; see also GETTLER 1927). So far, however, the observations concerning the occurrence of a secondary growth have not been convincing.

In all these species auxospore formation took place in great numbers, and although frequently examined no swarmers or microspores were ever found.

But, when reduction division and sexuality are connected with auxospore formation, what place in the life cycle of centric diatoms is taken by the microspores? The formation of cells resembling stages in the formation of microspores as described by various authors from plankton catches was only found in *Chaetoceros didymus* and *Ch. pseudocrinitus*. There is, however, reason to assume that these bodies, although containing a nucleus and chromatophores at first, do not take part in the reproductive cycle of the diatoms but represent stages of a peculiar degenerative process (see p. 33). The following observations may be recorded in connexion with the problem of microspores. On 20 December 1935 a raw culture of various diatoms, among them *Coscinodiscus Granii*, was made by inoculating a Petri dish with a small sample (about 1 c.c.) of plankton from a very fine-meshed tow-netting. On 3 January the culture was examined and several shells of *C. Granii* were found containing numerous motile flagellates, 10–12 μ long, with a distinct brown chromatophore at the base of the cell. Three such *C. Granii* cells were washed and isolated in a watch-glass. On 5 January a number of flagellates were found swimming outside the shells. During the following days most of them had left the shells, and the number of flagellates in the watch-glass seemed to be much larger than that contained previously by the three shells. However, bacteria had developed in great numbers and on 14 January the free-swimming flagellates had disappeared. In the diatom shells a few were still present but had lost their motility.

No doubt the flagellates had nothing to do with the life cycle of *C. Granii*. Evidently the diatoms in the raw culture had reproduced very rapidly during the first week or so, and so did the flagellates which were introduced into the culture with the plankton sample. It is a common experience of all workers who try to obtain pure cultures of marine diatoms that nanno-planktonic flagellates present in a diatom culture inhibit the growth of the diatoms. It seems very probable that the *Coscinodiscus* cells mentioned above had died in the crowded raw culture, that the cell content had disintegrated, and that one or few flagellates present in the raw culture found their way into the shell where they stayed and divided until all organic substances were exhausted. Then they gradually left the shell and lived in the surrounding medium until the strong contamination with bacteria made an end of them.

These observations offer an example of how various flagellates might be mistaken for microspores or microgametes. The possibility that some of the microspores described were in fact parasites has been often discussed (GERTLER 1931; FRITSCH 1935). Where the formation of microspores was preceded by nuclear divisions of the diatom cells (GRAN 1902, 1904; KARSTEN 1904, 1907; BERGON 1907; SCHILLER 1909; HOFKER 1928) the simple assumption of parasitic infection is clearly unsatisfactory.

My own observations point to the possibility of interpreting the great diversity exhibited in the observations of various authors with regard to the formation, the

ultimate shape and the supposed fate of the microspores as being due to two phenomena: (a) an abnormal division process similar to that found in *Chaetoceros didymus* and *Ch. pseudocrinitus* (p. 33), resulting in the formation of a number of small naked cells within the parental shell; (b) the occasional presence and reproduction in dead diatom cells of flagellates not necessarily of parasitic nature. This would lead to the view that what have been described as microspores are either abnormal products of the diatom cell or alien flagellates, neither participating in the life cycle of the diatoms.

This rather radical view, tentatively put forward, may be based on the following considerations. From the observations on cultures one can see that microspores do not take part in auxospore formation. There is very little evidence that they represent gametes, and there is accumulating evidence for the assumption that an autogamic sexual process is connected with auxospore formation. If, however, they were asexual spores directly developing into normal vegetative cells it would be very unlikely that such extremely competent observers as GRAN, KARSTEN, BERGON and others had missed the various stages of their development.

The number of microspores recorded in cells of different species varies from eight in *Melosira varians* (SCHMIDT 1923) to 128 in *Rhizosolenia styliiformis* (GRAN 1902). Another difference in their formation in different species is of interest. In diatoms where up to 128 microspores were recorded (*Rhizosolenia styliiformis*, *Corethron Valdiviae*) the nucleus underwent first a multiple division and only in a late stage the nuclei appeared surrounded by a protoplasmic film. In other forms the microspores were formed as in *Chaetoceros pseudocrinitus* by successive divisions of the nucleus and the protoplasm.

SCHILLER (1909) found two types of microspores, spherical ones, 2–3.3 μ in diameter, and oval ones of 5 μ length, which he regarded as macro- and microgametes respectively. Both types had no flagella and were non-motile. BERGON (1907), who has studied the microspores of *Biddulphia mobiliensis* most carefully throughout five years, described the formation and liberation of microspores possessing two flagella. He was unable to establish when the flagella were formed; he assumes that their development takes place between the stage of 16 and 32 cells. When the microspores had formed flagella further reproduction took place within the diatom shell. After they got out of it they swam about for a few days, then became motionless and died. They never copulated and therefore BERGON did not regard them as gametes but as asexual swarmers. It may be pointed out that the microspores exhibited strong differences in different years with regard to their shape and movement. One year they were spherical and showed rotating movement, another year they had an irregular oval shape or were pointed at one pole and their movement was more oscillating.

In view of these differences one is tempted to suppose that BERGON put two types of *Biddulphia* cells into one developmental series: cells which for some reason had abnormally formed 16–32 cells—corresponding to those found in *Chaetoceros didymus* and *Ch. pseudocrinitus*—and cells containing flagellates—corresponding to the *Coscinodiscus Granii* cells in my raw culture mentioned above.

With regard to the conditions for auxospore formation the centric diatoms dealt with in this paper show a similar behaviour to that of the Pennatae studied by GERTLER (1932). In both groups only cells of a certain range of diameter are capable of auxospore formation.

In the Pennatae it seemed that once the right size has been obtained the sexual reproduction would take place under optimal culture conditions without any changes of environmental factors. Thus the diatoms appeared to be the only exception to the rule, first conceived clearly by KLEBS, that in Protozoa and Algae sexual reproduction is induced by definite changes in environmental conditions. However, GERTLER found that if *Navicula seminulum* is cultured on alkaline Knop-agar with addition of 1% NaCl normal growth, division and decrease in size will take place but the sexual reproduction—fusion of gametes and auxospore formation—is completely suppressed, in spite of the fact that they were of a size which would permit auxospore formation. When transferred on Knop-agar without the additional NaCl they readily formed gametes and auxospores. These and experiments with low light intensity with similar results show that apart from the "internal factor", a certain cell size, certain environmental conditions must be realized before sexual reproduction can take place, but these conditions do not seem to differ from the optimal conditions for growth and asexual reproduction. The better the conditions for growth and division the more auxospores were formed.

For the centric diatoms it could be shown that the optimal conditions for growth and division on the one side and for auxospore formation on the other are different. During a period of several months after the cells had obtained the right size auxospores were formed by *Ditylum*, *Chaetoceros didymus*, *Skeletonema* and *Melosira* only in old cultures, where the medium had undergone certain changes and the rate of division had decreased. The degree of these changes necessary for inducing the formation of auxospores became smaller with a further decrease of the cell diameter, but only when the diatoms had reached a size which sets a limit to further asexual reproduction by division were auxospores formed in the old medium, as well as immediately after having been placed in fresh culture medium, thus resembling the behaviour of the Pennatae. It was shown that cells of the same culture of *Ditylum* formed auxospores much sooner in pure sea water than in sea water enriched with nitrate, phosphate and soil extract ("Erdschreiber"). In the centric diatoms the causation of auxospore formation by external, environmental factors is thus much clearer than in the Pennatae.

Resting spores only occur in centric diatoms. HENSEN (1887) and SCHÜTT (1888) found that the appearance of resting spores in plankton diatoms is an indication that they will soon disappear from the plankton. The resting spores sink slowly towards the bottom; in greater depths they were found to be more numerous than near the surface. GRAN (1902) pointed out their biological importance and opposed the views of CLEVE who tried to explain the seasonal variation of planktonic diatom populations

exclusively on the basis of currents, without considering the possibility of their reappearance from the sea bottom.

A discussion of the literature on seasonal variations in the abundance of planktonic diatoms and their causes deduced by various authors from analyses of the hydrographic and chemical conditions would fall beyond the scope of this paper. The observations and experiments on cultures described above have a bearing on those plankton problems only so far as resting spores are concerned.

It was found that apart from the presence of great quantities of bacteria three factors are responsible for the formation of resting spores in *Ditylum*. (1) "Overcrowding" of the culture, i.e. changes in the medium caused by the growth and reproduction of many diatoms. Some evidence was produced for the assumption that the growth of a culture to about 1000 cells per c.c. of culture fluid is a preliminary condition for resting spore formation. (2) Low temperature. (3) Low light intensity. The "overcrowding" factor has no effect at high temperature and strong light intensity—no resting spores were formed in the cultures during summer. Low temperature and dim light have no effect in young cultures with less than about 1000 cells per c.c. of culture medium.

At this degree of overcrowding during autumn and winter, the cultures often produced thousands of resting spores overnight when the temperature dropped and the resting spores germinated during the following day when the temperature rose again. The changes of the culture medium were sufficient to induce resting spore formation at low temperature and low light intensity but not advanced enough to prevent germination immediately after the disappearance of these two factors. After a few days the higher degree of overcrowding resulted in the production of persistent resting spores which germinated only when transferred into fresh culture medium. For germination fresh culture medium, i.e. the absence of the "overcrowding" factor, is of almost exclusive importance. It takes place in fresh culture medium under otherwise the same conditions which induce the formation of resting spores in old cultures.

How can these observations be applied to the conditions in the sea? The growing diatom population during the spring and autumn outburst can certainly be compared with a growing culture, and there is enough evidence for the fact that the overcrowding of the surface layers of the sea results in the exhaustion or, at any rate, a considerable decrease in the amount of nitrates and phosphates. The same may be true for other substances which have not yet been studied quantitatively. It may well be that the exhaustion of some substances during the outbursts is partly responsible for the formation of resting spores just as in the cultures. It has been pointed out before (p. 19) that the degree of "overcrowding" in the sea, i.e. the density of the plankton population, need not be as high as in the cultures to be equally effective.

It appears, however, difficult to compare the two other factors in their effect upon the plankton and the culture populations respectively. "In the Channel... *Ditylum*

Brightwelli is a perennial species, but has, however, a distinct flowering period in the winter half-year and a pronounced depression in the summer" (OSTENFELD 1913). It occurs in Plymouth waters mainly during autumn and winter. It may take part in the spring outburst of diatoms in March, and shortly afterwards disappear from the plankton (HARVEY, COOPER, LEBOUR and RUSSELL 1935). If the disappearance were due to resting spore formation this process would appear to take place under increasing light intensity and temperature as compared with the conditions in winter—contrary to the experience with cultures. However, the water temperature in March is well below 10° C. and thus sufficiently low for resting spore formation. With regard to the light intensity there is another possible interpretation of the conditions for the production of resting spores in nature which would bring them in accordance with the observations on cultures.

Some of the cultured species, *Chaetoceros didymus*, *Ch. pseudocrinitus*, *Coscinodiscus excentricus*, *Skeletonema*, *Streptotheca* and *Thalassiosira*, are freely suspended in the cultures. As soon as these become crowded they sink to the bottom. *Ditylum* is not suspended to the same extent as the other species just named. The cells are lying mostly at and near the bottom but in young cultures slight shaking, e.g. by placing a Petri dish culture under the microscope, is sufficient to upset their position and to lift many cells towards the surface, whereas in old cultures their position at the bottom of the glass dish gets less easily disturbed.*

If in the sea, as in cultures, the diatoms tend to sink when overcrowding occurs, and if with overcrowding in the upper water layer their capacity for suspension were reduced, the cells would gradually sink down and reach water levels with lower light intensity, and perhaps with lower temperature, than at the surface. Here the actual formation of resting spores would take place. The same factors which in a culture induce *Ditylum* cells to undergo resting spore formation would in nature act in a somewhat different way. The temperature being sufficiently low for this process to take place, the overcrowding factor is responsible for the sinking of the cells and their passing through water layers with light conditions which favour the formation of resting spores. As we have seen, their germination in cultures can take place in almost complete darkness and at comparatively low temperature, provided the cells are placed in fresh culture medium. The same conditions may prevail in the sea. The resting spores would remain suspended in a deep-water layer or lie at the bottom until the sea water becomes enriched with those substances which probably became exhausted by overcrowding.

The validity of certain conclusions drawn above may be tested on some interesting results of plankton investigations by LOHMANN (1908). This author found for *Chaetoceros*

* We have very little knowledge of the mechanism which enables diatoms to remain suspended. KARSTEN (1907) draws attention to the possibility that there may exist in some diatoms, particularly in those without long bristles or spines which increase the resistance towards sinking, as in *Coscinodiscus*, a similar storage of CO₂ in the vacuole fluid for the regulation of suspension as was found in *Radiolaria* by BRANDT. There is evidence for the same mechanism being responsible for the floating of *Noctiluca* (GROSS 1934).

that during the spring and autumn outburst, as soon as these diatoms reached their maximum number, resting spores occurred in the plankton.

On the whole the vegetative growth and reproduction during the spring outburst takes place almost exclusively in the surface layer of 0–5 m. Towards the end of this period, when the number of vegetative cells decreased in the uppermost layers, many vegetative cells and resting spores occurred at 5 m. and finally many vegetative cells and enormous numbers of resting spores—up to 14,000,000/100 l.—at 15 m. depth.

The facts that in the sea *Chaetoceros* cells start sinking when overcrowding of the plankton sets in and that the main bulk of resting spores is formed at 5–15 m. depth, i.e. in a layer of lower light intensity and possibly lower temperature, seem to support the conclusions drawn from the experiments on cultures.

A perfect parallel to LOHMANN's observations was recorded by GRAN (1915). This author found in the North Sea on a certain day in May that the diatoms were distributed vertically throughout the whole of the water mass. The maximum production had passed and a great number of resting spores of *Chaetoceros debile* and *diadema* were found throughout the water column, but in particularly great quantities in the deep water. There were of the first-named species at 0 m. 1580 vegetative cells and 12,400 resting spores per litre, at 68 m. depths 6500 and 195,100 respectively.

The conditions for resting spore formation are probably different, at least in degree, in different species of diatoms, as are the optimal conditions for reproduction in the sea. The occurrence of a number of resting spores at 0 m., as found by LOHMANN in *Chaetoceros*, if not due to currents which had brought them up from deeper water layers, might not perhaps be found in *Ditylum* which, as LOHMANN states, was only found in September, October and November, at first and for the longest time in a depth of 15 m.

The connexions drawn above between observations on cultures and those on plankton are only of a preliminary nature. But it seems certain that it would be profitable in future plankton studies concerned with the abundance of diatoms in different seasons to pay more attention to resting spores and auxospores. Their occurrence and distribution might turn out to be an indicator of some definite changes in the chemical and hydrographical conditions of the corresponding waters. In work on cultures concerned with the chemical and physical conditions for the growth of diatoms it would appear equally profitable not to concentrate on the division rate only but to analyse the conditions for the formation and germination of resting spores respectively, and the formation and development of auxospores.

SUMMARY

A number of centric plankton diatoms have been kept in pure cultures in FÖYN's "Erdschreiber" as culture medium. The cultures were started with one or few cells isolated from plankton samples and washed repeatedly in sterile medium.

Most observations and experiments were made on *Ditylum Brightwelli*. In this species the cell division is remarkable because of a wide space formed between the daughter cells before their separation. The formation of new valves is not entirely dependent on cell division (see secondary valves, p. 7).

The mitotic division has been found to be very similar to that of other Algae. Neither centrosomes nor an extranuclear origin of the spindle could be observed.

Resting spores occurred regularly and in great numbers in *Ditylum* cultures during autumn, winter and early spring, not, however, in summer. They are spherical bodies formed inside the parental shell by contraction of the protoplast. Their membrane becomes silicified.

The interaction of three factors was found to be responsible for the formation of resting spores. (1) Certain changes of the culture medium, probably due to the exhaustion of some substances, gradually taking place with the growth of the culture. Except in a few cases of heavy contamination with bacteria no resting spores were formed unless the culture reached a density of about 1000 cells per c.c. of culture medium. (2) Low temperature. Most resting spores were formed over night when the room temperature dropped to 10° C. and below. When rich cultures which have not yet produced resting spores at room temperature are placed in an ice-box, almost 100 % of cells will form resting spores within a few hours. (3) Low light intensity. In summer low temperature has very little effect on overcrowded cultures with regard to the formation of resting spores, unless the cultures are placed in dim light for some time before the exposure to cold. Low light intensity alone does not induce resting spore formation.

Before the density of the cultures reaches such a degree that persistent resting spores are formed, their formation in autumn and winter takes place for some time overnight, followed by germination in the course of the next day. Persistent resting spores germinate only if transferred into fresh culture medium, and then under otherwise the same conditions which induce their formation in old cultures. Their germination may take 3-12 and more days.

At the beginning of the germination the resting spores expand slightly and fine protoplasmic filaments are sent off towards the original cell membrane. These connexions become stronger, and by gradual elongation and expansion of the protoplast the cell gains normal appearance. The formation of resting spores is almost exactly the reverse process.

A continuous decrease of the average cell diameter could be observed in cultures of *Ditylum Brightwelli*, *Chaetoceros didymus*, *Skeletonema costatum*, *Melosira Borreri*, *Chaetoceros pseudocrinitus*, *Coscinodiscus Granii*, *C. radiatus* and *C. obscurus*. In the first four species the formation of auxospores was studied, a process by which a broad cell diameter is restored. When the diameter of the diatoms reaches a certain minimum size no further growth or division is possible and they perish unless they form auxospores which develop into big cells. The maximum and minimum diameters respectively were

found to be 100 and 10μ in *Ditylum*, 26 and 4μ in *Chaetoceros didymus*, 12 and 2μ in *Skeletonema*, 19 and 10μ in *Melosira*. The average length of the cells may vary considerably in successive subcultures, but no progressive decrease or increase takes place corresponding to the steady decrease of the diameter.

Auxospores are the only stages in the life history of diatoms which are capable of growth along all axes. It is by means of these special cells that the cell diameter of a diatom population is restored. They are spherical bodies formed by the outflow of the protoplast of cells grown to a length greater than that at which otherwise cell division would occur. Auxospore formation only takes place in diatoms of a certain diameter, not larger than 45μ in *Ditylum*, 8μ in *Chaetoceros didymus*. Once this preliminary condition is realized, auxospore formation may be induced by environmental factors; the main factor being again progressive changes of the culture medium due to the growth of the culture population. Comparatively broad cells only form auxospores in old, very dense cultures, and only very few if any of the auxospores will develop into broad cells unless transferred into fresh culture medium. The smaller the cell diameter the more ready become the cells to form auxospores. The degree of overcrowding necessary to induce auxospore formation becomes much smaller in narrow cells and the auxospores develop into broad cells which reproduce side by side with the cells of the original size.

Cells from the same culture will form auxospores much sooner in pure sea water than in "Erdschreiber". They will, however, not develop into broad cells unless transferred from sea water into "Erdschreiber".

Broad cells develop from newly formed auxospores in 2 days, from older ones in 3-7 days.

Cytological observations on *Ditylum Brightwelli*, particularly the fact that young auxospores were found containing one big and two small nuclei, give support to PERSIDSKY'S view that reduction division and an autogamic sexual process precede auxospore formation. No microspores take part in this process.

Intracellular bodies resembling microspores as described by several authors were only found in *Chaetoceros didymus* and *Ch. pseudocrinitus*. They are interpreted not as reproductive cells but as products of an abnormal cell division.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1—*Ditylum Brightwelli*. *a*, growing cell; *b*, cell after division; *c*, one daughter cell after separation. *a*, *b*, *c* from the same culture. *d*, a broad cell developed from an auxospore after division. Acetic-carmin. $\times 520$.*

FIG. 2—Some division stages of a *Ditylum* cell. The corresponding time given on each figure. $\times 250$.

FIG. 3—Some division stages of a narrow *Ditylum* cell. $\times 250$.

FIG. 4—Some stages of the nuclear division of broad cells (Axp.-line). *a*, resting nucleus; *b*, late prophase, optical section; *c*, *d*, metaphase, *d*, side-view, optical section; *e*, telophase; *f*, interphase. Acetic-carmin. $\times 1400$.

FIG. 5—Some stages of the nuclear division of narrow cells (line A and C). *a*, resting nucleus; *b*, early, *c*, late prophase; *d*, metakinesis; *e*, late anaphase; *f*, telophase; *g*, daughter nuclei. Acetic-carmin. $\times 1400$.

* This and all the subsequent figures were drawn with the aid of a camera lucida.

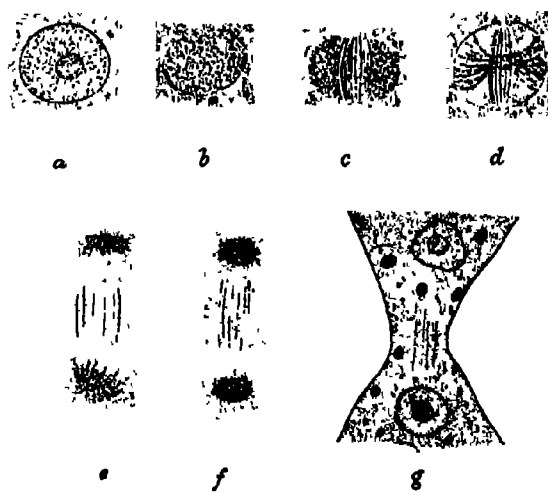
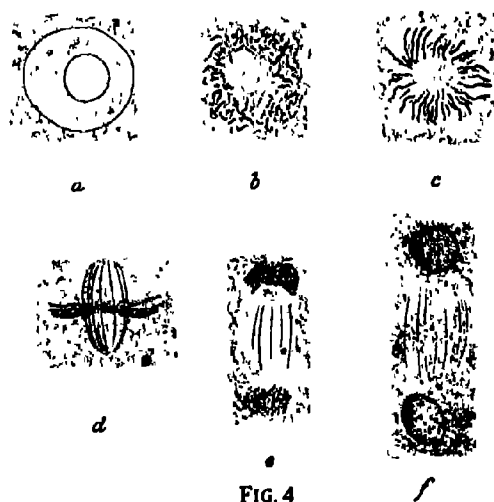
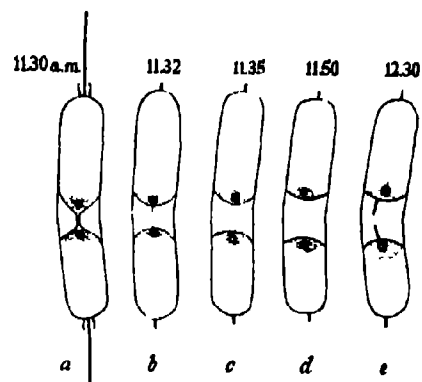
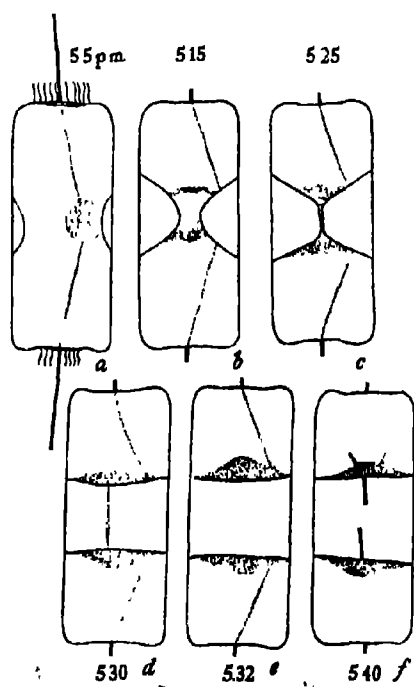
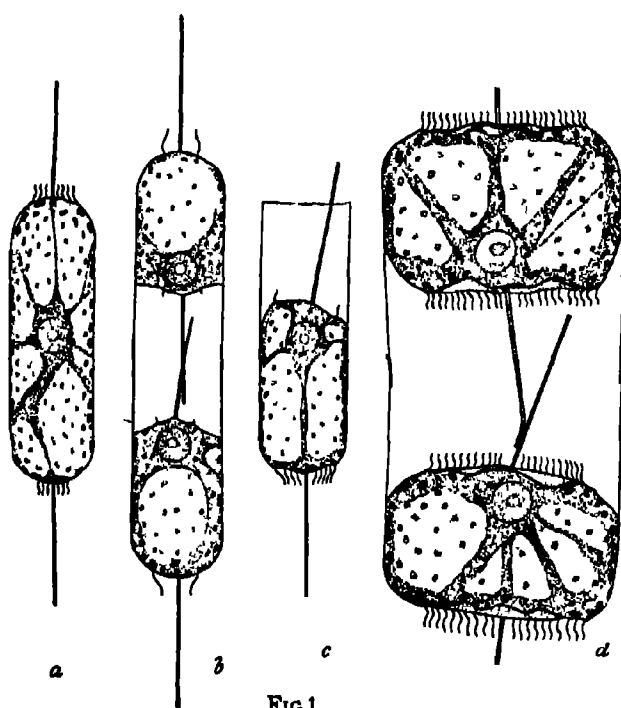


PLATE 2

FIG. 6—Secondary valves. *a*, from the same culture as fig. 1 *a-c*; *b*, resting spore with five secondary valves. *a* $\times 560$; *b* $\times 180$.

FIG. 7—*a*, cell after division; *b*, one daughter cell of *a* with a secondary valve; *c*, the same after having grown and pushed off the primary valve. Below the figures the dates at which they were drawn. $\times 350$.

FIG. 8—*a*, resting spore; *b-d*, stages of germination of *a*; *e*, the same cell after division. $\times 350$.

FIG. 9—*a*, resting spores formed in a cell short time after division; *b*, first stage of germination. $\times 350$.

FIG. 10— Some stages of germination of a resting spore formed by a broad cell. $\times 250$.

FIG. 11— Germination stages of a sister cell of that on fig. 10. $\times 250$.

FIG. 12 - Four stages of the formation of resting spores (cells from the same culture). $\times 250$.

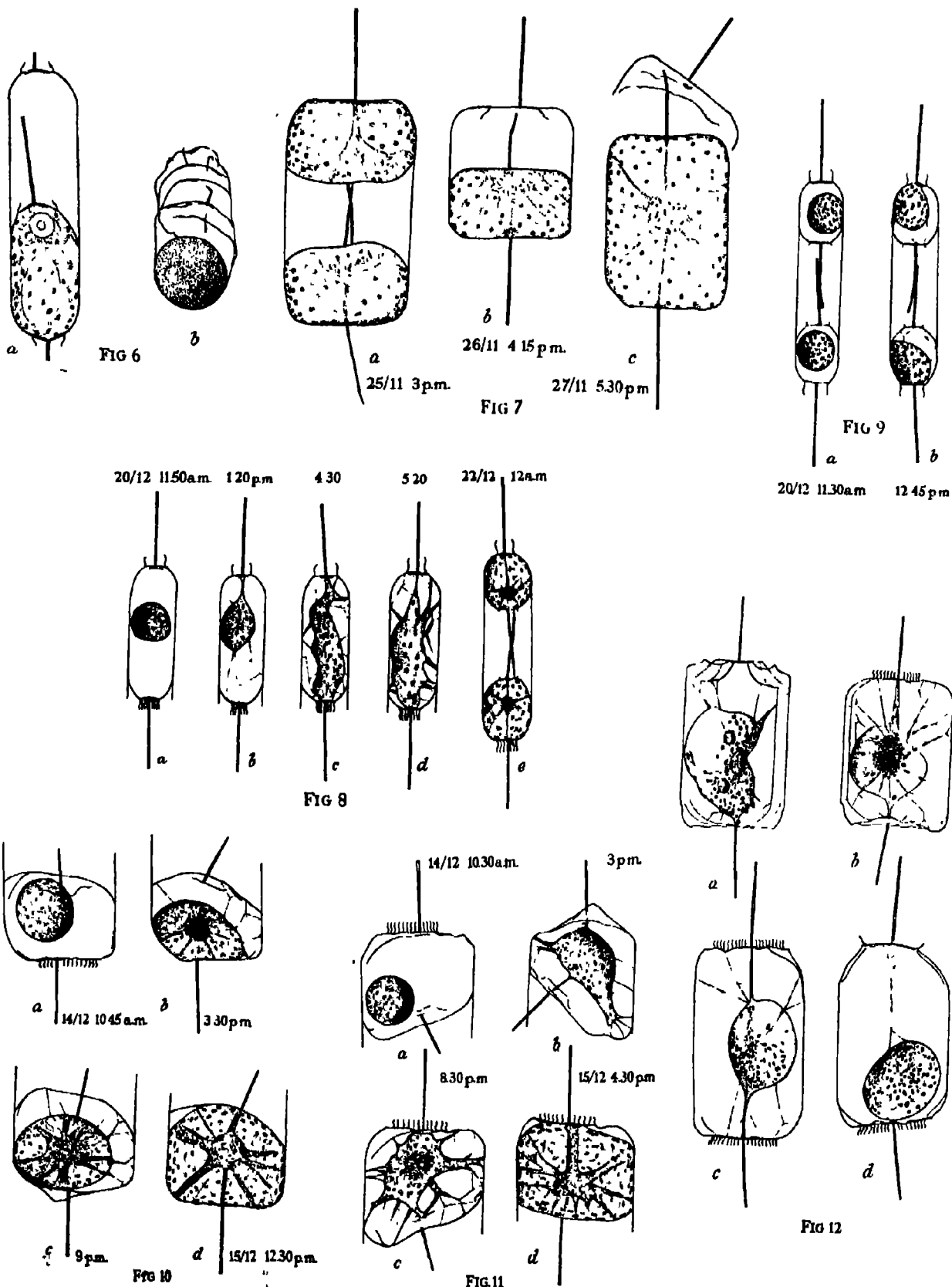


PLATE 3

FIG. 13—*a, b*, two cells isolated from the plankton, probably germinating resting spores; *a*¹, *b*¹, the same cells next day. × 250.

FIG. 14—*a*, an irregular resting spore formed after exposure to low temperature (4 December 1935); *b*, the same after germination (9th). × 250.

FIG. 15—*a*, irregular resting spore formed under the influence of bacteria; *b*, the same immediately after transference into fresh culture medium; *c*, the same four days later, germination almost completed. × 250.

FIG. 16—*a*, cell undergoing auxospore formation; *b*, completed auxospore. × 350.

FIG. 17—Auxospore formation. *a* and *b* as in fig. 16. × 170.

FIG. 18—Stages of development of one auxospore into a broad cell. × 250.

FIG. 19—*a*, outline of the nucleus of a narrow cell, *b*, of a broad cell from the same culture, developed from an auxospore; *c, d*, stages of auxospore formation, *c*, containing two nuclei, *d*, one big and two small degenerating nuclei; *e*, young auxospore with one big and two small nuclei. Acetic-carmin. *a, b* × 980; *c-e* × 650.

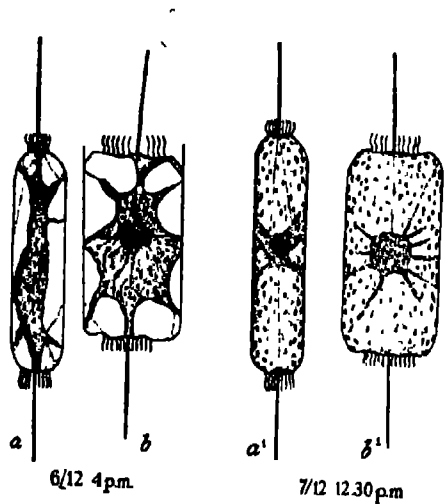


FIG 13

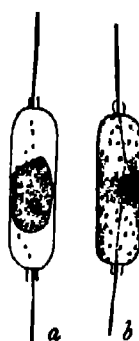


FIG 14

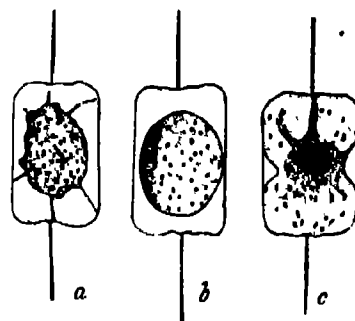


FIG 15

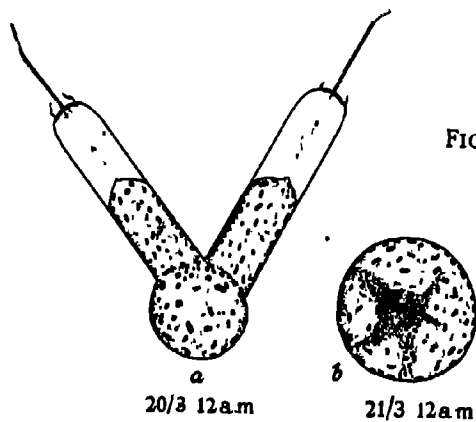


FIG 16

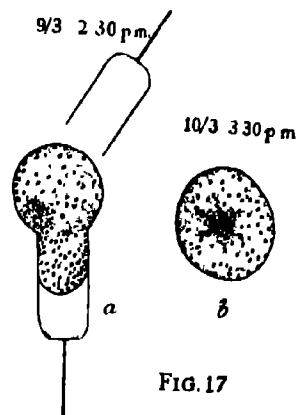


FIG. 17

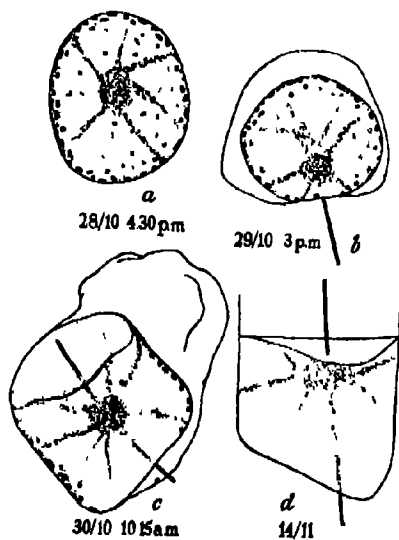


FIG. 18

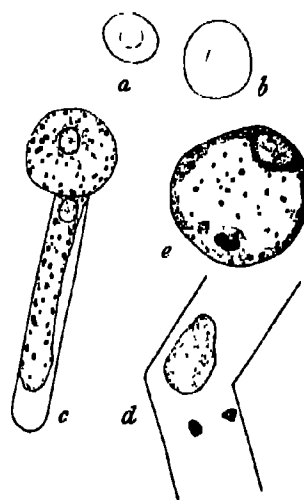


FIG 19

PLATE 4

FIG. 20—*Chaetoceros didymus*. *a*, part of a chain of narrow cells, *b*, of broad cells developed from an auxospore in the same culture. $\times 250$.

FIG. 21—Resting spores of *Chaetoceros didymus*. $\times 250$.

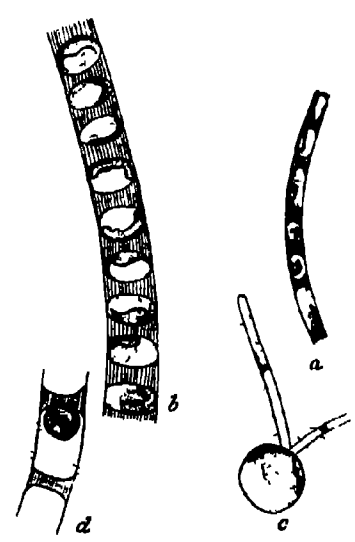
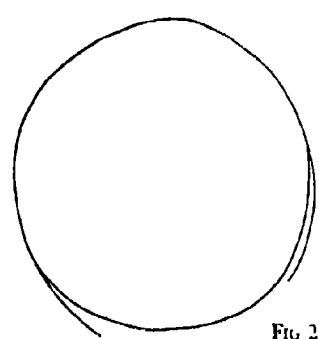
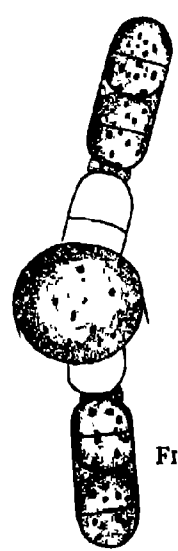
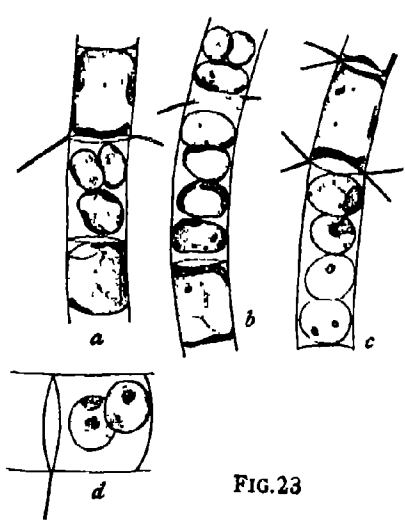
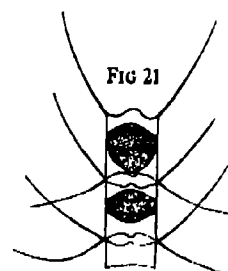
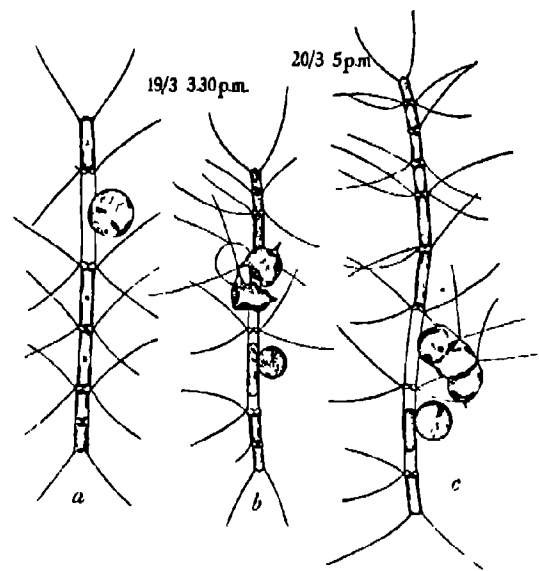
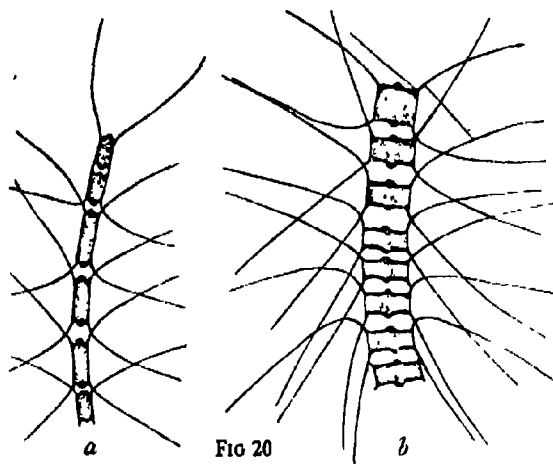
FIG. 22—*a*, chain containing a cell which had formed an auxospore; *b*, chain with two broad cells developed from an auxospore, one cell (below) undergoing auxospore formation; *c*, the same next day. $\times 250$.

FIG. 23—Microspore-like bodies in cells of *Chaetoceros pseudocrinitus*. *a-c*, from living cells, $\times 920$. *d*, acetic-carminc, $\times 1400$.

FIG. 24—*Skeletonema costatum*. *a*, part of a chain of narrow cells, *b*, of broad cells developed from an auxospore in the same culture; *c*, auxospore; *d*, resting spore. $\times 560$.

FIG. 25—*Melosira Borreri*. Auxospore with newly formed epitheca. $\times 930$.

FIG. 26—Outline of a *Coscinodiscus obscurus* cell with burst connecting band. $\times 250$.



II—THE ACANTHODIAN FISHES

By D. M. S. WATSON, F.R.S., *Jodrell Professor of Zoology and Comparative Anatomy, University College, London*

(Received 22 December 1936—Read 29 April 1937)

[PLATES 5–14]

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INTRODUCTION

The Acanthodian fishes form one of the most sharply demarcated and recognizable groups of vertebrate fossils. Their characteristic squamation of square, exceedingly minute ganoid scales, and the fact that all the fins except the caudal fin are supported by large anterior spines, distinguish them sharply from all other fishes, and enable even fragmentary specimens to be recognized without doubt. They are worldwide in distribution, and their range in time is thus known with considerable certainty.

The first fragments, attributed on very good evidence to the group, are isolated fin spines, found in Upper Silurian rocks perhaps not earlier than the Downtonian, which seem to be identical with those found in complete fishes of Lower Devonian age.

The group was more varied in structure and played a larger part in the world in Lower Devonian times than at any other period. Even in the Middle Old Red Sandstone the range in structure had been reduced, and by Carboniferous times very few forms remained.

Finally the group disappears during the Lower Permian, occurring, essentially in the form of a single species, in the Rothliegende of Europe.

Thus the Acanthodians were the earliest group of vertebrates, apart from the Ostracodermi, to reach their maximum and decline, antedating all certain Elasmobranchs and all bony fishes, and even the Arthrodeirs and Antiarchi. They have therefore a special interest because now it is known from the work of Professors STENSIO and KÆR that the Ostracodermi are related to the lampreys, the Acanthodians become the most archaic and earliest of gnathostomes, and their morphology, when fully understood, may be expected to throw light on all the various problems connected with the formation of the jaws and the backward migration of the mouth which is associated with them.

Material representing Acanthodian fishes is locally abundant at all periods from the Lower Devonian to the Permian, but very little of it is sufficiently well preserved to display much of the structure of the head. The fishes from the Lower Old Red Sandstone of Turin Hill and Farnell in Forfarshire are often perfectly preserved so far as their external surface is concerned, but they give exceedingly little evidence as to the character of the endoskeleton, either of the head, trunk or fins.

The Middle Old Red Acanthodians from the nodule localities of the Moray Firth are sometimes beautifully preserved, but they also are crushed flat, and consequently the traces of neural cranium visible in them are unintelligible.

Specimens of *Acanthodes* from the Coal Measure Ironstones may be little crushed and well preserved, but they are remarkably rare and I have been able to do little with them.

There remains therefore only "*Acanthodes bronni*", which is common and in many ways exceedingly well preserved in the Ironstone nodules from the Lebach shales near Saarbruck. This fish is indeed the only Acanthodian the structure of whose head can be determined at all completely, but as it is the last surviving member of the group it is not necessarily representative of the structure of the majority of the genera, although it must conform to the underlying morphology of the group.

The literature dealing with Acanthodians is exceedingly extensive. Beginning with the foundation of the genus *Acanthodes* by AGASSIZ in the "Poissons Fossiles", they have been dealt with by nearly every author who has concerned himself with fossil fishes, but few have added materially to our knowledge of the structure of any member of the group. ROEMER (1857), KNER (1868), POWRIE (1864, 1870), TRAQUAIR (1894), and SMITH *WOODWARD (1891) are the most important references for our general knowledge, otherwise than of the head.

The head skeleton of *Acanthodes bronni* was described by O. M. REIS in a series of papers, of which the latest and most important are two published in 1895 and 1896. One of them (1895) consists of a series of plates containing admirable and, in the only case in which I have made direct comparison with the original, scrupulously accurate drawings of the head. The other (1896) contains a detailed description and discussion

of the morphology of the whole skeleton. This paper has never received the attention that it deserves because it is exceedingly difficult to follow without actual material at hand, and it contains one or two suggestions which cannot be supported; but it is in general, so far as I can claim to understand it, accurate as to its facts, as distinguished from the interpretation sometimes placed on them. The only two authors who seem seriously to have considered REIS's papers are JAEKEL and BASHFORD DEAN. The former made a series of magnificent preparations of the material of *Acanthodes bronnii* in Berlin and elsewhere, but never gave a connected account of the structure which they showed, contenting himself with partial drawings and descriptions scattered throughout papers dealing with very different subjects over a period of a quarter of a century, and indulging in occasional polemics with REIS. Professor BASHFORD DEAN (1907) examined some of Professor JAEKEL's material and gave, in a short paper in the *American Journal of Anatomy*, a discussion of certain parts of the anatomy of "Acanthodian sharks" which contained many important observations and criticisms of the views of REIS, JAEKEL and SMITH WOODWARD, together with some admirable drawings illustrating anatomical details.

Thus, despite the extent of the literature, our real knowledge of the Acanthodians is limited in extent, and scarcely justifies the certainty with which these fishes have been referred to the Elasmobranchii, or to some other position in the class Pisces. Acanthodian material is abundant; in connexion with this paper I must have examined about 1000 specimens, but of these very few are of value for the study of the morphology of the head. The general absence of dermal bones which are individually recognizable, the exceedingly small size of all the scales and head plates, and the fact that when crushed flat, as with very few exceptions all Acanthodians are, the two surfaces of the head become so closely pressed on to one another that it is difficult to distinguish their structures no matter how well the individual bones may be preserved, probably account for the fact that the Acanthodians are now the only group of primitive fishes of whose morphology we have no detailed knowledge.

The method of investigation I used is as follows.

The head of the specimen was photographed with carefully considered lighting and often through a coloured screen, and an enlargement at a magnification of from 3 to 10 diameters was made from the negative.

Miss JOYCE TOWNEND then inked in on the enlargement the borders of all the individual bones visible in the specimen when examined under a fairly high-power (30 diam.) binocular microscope. I then discussed any doubtful points with Miss TOWNEND until we reached agreement.

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well-marked structure like Meckel's cartilage or a pectoral fin spine. By such comparisons additional details can often be added to the restoration. The whole process is exceedingly laborious, but it does give considerable certainty and leads to the discovery of many otherwise unobserved facts.

As no existing account of any Acanthodian is reasonably complete, accurate and intelligible, I have thought it well, in view of the great morphological importance of their structure, to give straightforward accounts of the structure (so far as it is known to me) of *Climatius reticulatus*, *Euthacanthus macnicoli*, *Brachyacanthus scutiger*, *Parexus incurvus*, *Mesacanthus mitchelli* and *Ischnacanthus gracilis* from the Lower Old Red Sandstone of Forfarshire, of *Diplacanthus striatus* and *Cheiracanthus* from the Middle Old Red Sandstone of the Moray Firth and of *Acanthodes* from the Upper Carboniferous Ironstones of Lebach in the Saar.

CLIMATIUS RETICULATUS

Climatius reticulatus is the third most abundant fish in the Lower Old Red Sandstone of Turin Hill, but is none the less an uncommon fish, contrasted with the numbers of *Mesacanthus* and *Ischnacanthus* to be found there.

The more important specimens are: B.M.N.H. No. 38596, and its counterpart Powrie Collection 1891, 92, 198 in the Royal Scottish Museum. This fish has been so preserved that the dorsal surface and right lateral surface of the head are seen on one slab, and the left lateral and ventral surfaces on the other. The dermal bones are displayed from their visceral surface and are in perfect condition.

L. 12096, A and B, Manchester Museum, is the most complete existing specimen and is valuable for the structure of the head, dentition, fins and body shape.

Powrie 1891, 92, 206, R.S.M., and its counterpart P. 6961, B.M.N.H., show the ventral surface of the head and shoulder girdle very well.

Royal Scottish Museum 1887, 35, 5, and counterpart P. 6961 a, B.M.N.H., are important for the shoulder girdle, the orbits and anterior end of the head.

No. 49785, Museum of Practical Geology, has a good palato-quadrate and Meckel's cartilage.

Powrie 1891, 92, 204, R.S.M., is the only well-preserved laterally compressed fish.

The majority of the specimens of this species are crushed dorso-ventrally as far back as the pectoral region, but the posterior part of the body is seen in direct lateral view. As a result of this condition the intermediate spines of the two sides, which lie between the pectoral and pelvic fins, fall on one another and become singularly difficult to count.

Head—No traces of the neural cranium are ever to be seen, and it is certain that it lacked completely all calcifications. The palato-quadrate and Meckel's cartilage are, however, usually calcified; they are never well preserved, and it is probable that the hardened layer was entirely superficial. It appears very finely granular, differing from

the bone of the scapula and from all the dermal elements in its texture. The material has not been investigated microscopically but is quite obviously not comparable with Elasmobranch cartilage calcification.

The jaws are best shown in No. 49725, Geological Survey, and in the Edinburgh specimen Powrie 206. The palato-quadrate is in principle like that of a dogfish; it has a deep posterior portion whose upper border is turned outward as a ridge but forms a smoothly curved margin which gradually sinks to the shallow anterior end, where it is cut out into two shallow bays. It is certain that there is no otic process homologous with that of *Acanthodes*, *Heptanchus*, etc.

Meckel's cartilage appears to extend forward to meet its fellow at a place a little in front of the anterior extremity of the palato-quadrate. It has a typical Elasmobranch appearance with a deep rounded posterior part, becoming shallower anteriorly but remaining rather massive to its end.

There are no teeth visible in the upper jaw but many specimens show the lower dentition, always very imperfectly because of its remarkable nature. The lower dentition seems to extend uninterruptedly and without any recognizable change of character from a point half-way along the length of the jaw, round a semicircular symphysis to the corresponding point on the other jaw. It is throughout composed of fused whorls of teeth whose bases coalesce to form a smooth cylindroid surface which was attached to the oral margin of Meckel's cartilage. The whorl seems to consist of at least three teeth. The individual teeth are most curious; each is thin in proportion to its width, and unexpectedly high. Its free extremity is broken up into three or five needle-pointed cusps by grooves on each surface of the tooth. These teeth are in detail quite unlike any others with which I am acquainted; in particular they do not in the least recall those of Elasmobranchs, although of course the whorl is in principle similar to the dentition of these far more recent fish.

The only other cartilages visible in the head are a crushed series of cerato-hyals, and probably two pairs of cerato-branchials seen from below in Powrie 206. They appear to be unsegmented and are so badly preserved that they cannot be further described.

The Dermal Bones of the Head—The whole external surface of the head is covered with an irregular mosaic of small polygonal ossicles, each one of which has a flat inner surface, whilst its outer surface is raised into a series of one or more denticles, each having its surface sculptured into a radiating series of angular ridges and grooves.

The dorsal surface of the head is perfectly shown from its inner side in No. 38596, B.M.N.H. In this specimen the normal regular squamation of the dorsal surface of the trunk suddenly passes over to a much more irregular arrangement of minute bones which in the mid-region of the head are no larger than ordinary scales. Farther to the side these bones gradually become larger until, above the gill chamber, the surface suddenly terminates in a row of relatively large elements, each about 2 mm. long. The whole width of the top of the head between the margins of the gill chambers is covered

by about 16-17 bones. The marginal row of larger bones has attached to its lateral border a row of very narrow elements which perhaps turned downward to the inner surface of the gill chamber. At two neighbouring points the marginal row is produced downward on to the summits of the hyoid and first branchial arch.

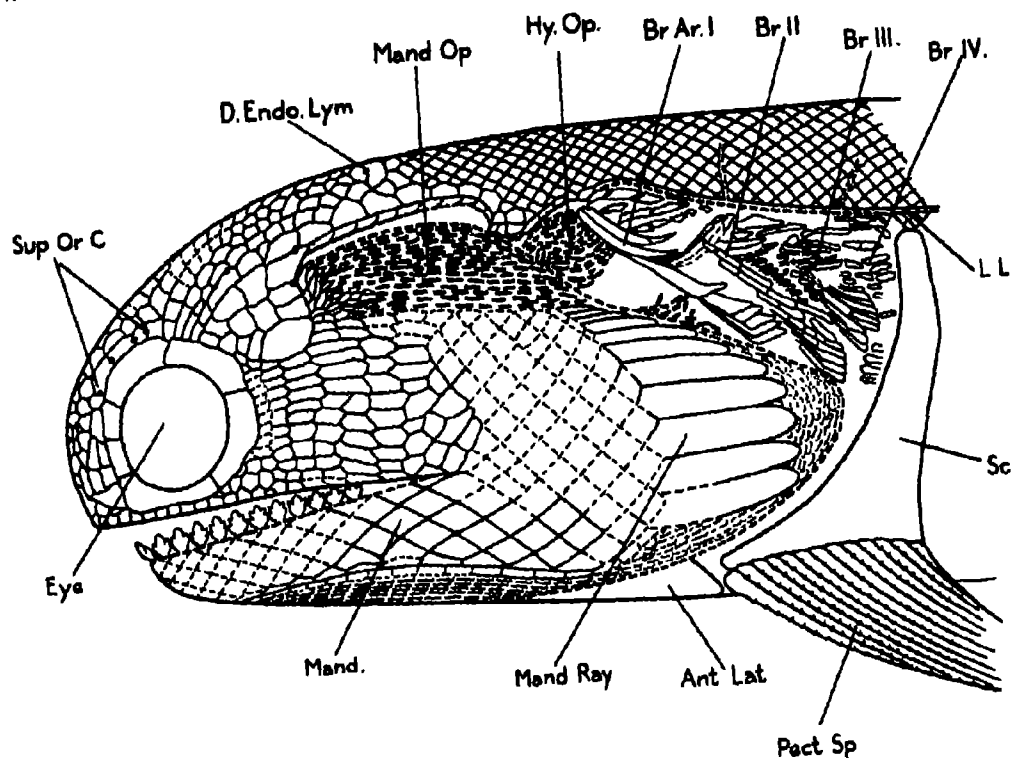


FIG. 1.—*Climatius reticulatus* AG. \times about 2.0. Reconstruction of the head founded almost entirely on the single specimen B.M.N.H. 38596 and Powrie 1891, 92, 198, from the L.O.R.S. of Turin Hill. All structures surrounded by continuous lines are accurate drawings from this specimen. *Ant.Lat.* antero-lateral dermal bone of the shoulder girdle; *Br.Ar. I*, *Br. II*, etc., the dermal elements of the branchial arches and their opercula; *D.Endo.Lym.* foramen for the ductus endolymphaticus; *Eye*, the orbit; *Hy.Op.* hyoid operculum; *L.L.* lateral-line; *Mand.* mandible; *Mand.Op.* mandibular operculum dorsal end; *Mand.Ray*, a "ray" in the mandibular operculum whose posterior margin is a thick broken line; *Pect.Sp.* pectoral spine; *Sc.* scapula; *Sup.Or.C.* supra-orbital canal.

There is only one recognizable irregularity in the roof of the skull in this region, the presence a little to each side of the mid-line of a pair of rather larger bones which meet one another in a straight transverse suture. On the left side in this suture there is a very minute foramen whose border lies mainly in the posterior bone; the opening is less clearly seen on the right. This foramen is no doubt for the ductus endolymphaticus.

The marginal row of bones of the temporal region is continued forward until it terminates in a large ossicle, separated by a single small element from the circum-orbital ring. The inter-orbital width is great, about as wide as the temporal region, and is covered with the ordinary polygonal ossicles, those which lie laterally being rather larger than the medial series.

The orbit is surrounded by a series of six large, massive bones, each of which has a smooth concave inner surface terminated by a low, rounded ridge which forms the actual orbital margin. The outer surface of each bone is very heavily ornamented with ridges which radiate from the centre of the orbit and bear series of low points. The free margins of this ring of circum-orbitals are attached by sutures of normal character to the surrounding dermal bones, so it is certain that the whole is not composed of sclerotic plates.

The anterior end of the head scarcely projects in front of the orbits and forms a smooth snout covered by irregular transverse rows each of about nine small bones. Only one specimen (Powrie 206) gives any evidence of nostrils; here on the left side there is a very small hole surrounded, at least so far as its dorsal border is concerned, by four radiately arranged elements. If this structure be correctly determined the two nostrils must have lain very close to one another and have been directed forward at a distance of about half the diameter of the orbit above the margin of the mouth. Other specimens, Edinburgh 1887, 35, 5 and Powrie 198, show this region well and make it certain that no nostril exists below this point, they also show no signs of a ventrally directed nostril opening below the ventral border of the dermal bones of the snout.

The mouth has no distinct borders. Its upper margin is quite indefinite, being marked only by the disappearance of the small dermal bones attached to the circum-orbital bones, the most ventral of which seems actually to be separated from the border by a single row of small bones. In the mid-line there is a larger though still very small bone which is symmetrical and bears a median knob.

Behind the eyes, and immediately below the large bone which marks the anterior end of the marginal series of the temporal region, lie a series of very narrow elongated bones which form horizontal rows. Posteriorly these pass gradually into a quite irregular mosaic of larger polygonal ossicles which extend downward without any break to the lower margin of the lower jaw behind the angle of the rather short mouth. In B.M.N.H. No. 38596 this area of the cheek has a very definite upper and posterior limit, which coincides with that of the palato-quadrate cartilage, and is probably co-extensive with Meckel's cartilage. This border lies parallel to, but is far removed from, the lateral border of the temporal region and its continuation on the anterior part of the body squamation above and behind the gill chamber.

The characters of the pharyngeal region are only shown quite satisfactorily in B.M.N.H. No. 38596 and its counterpart Powrie 198, but other specimens provide confirmatory evidence for most of the more important features.

The well-defined, clear-cut edge of the gill chamber is very obvious and implies the presence of an opercular apparatus and the depression of the gill-bearing region. The anterior part of the operculum (*Mand.Op.*) is well shown as a sheet of skin containing very small elongated ossicles lying in irregular horizontal rows, a little separated from one another. The whole structure is attached to the cheek along a line on the level of the free upper border of the hinder part of the palato-quadrates. This operculum is bounded anteriorly by a series of larger but still very small bones arranged in a sickle shape; the upper border is bounded by a single row of similar bones, pulled away from the margin of the temporal region. This operculum is shown definitely to pass laterally to the downward projection of the temporal region on to the upper end of the hyoid arch. Below this point the operculum is destroyed, its place being in part occupied by five small opercular (?) rays, all misplaced, and in some cases lying on the inner surface of the operculum; it is uncertain whether these elements belong to the series described below or are misplaced hyoidean bones. The next visible element of the operculum consists of a series of very large thick bony rays (*Mand.Ray*), whose long dorsal and ventral borders are in contact with one another. The anterior ends of these rays are joined to the posterior ossicles of the cheek by ordinary attachments without any sign of a definite movable articulation. It is quite evident from the whole material (especially Powrie 204) that these opercular rays are attached by their anterior ends to the outer surfaces of the articular ends of the palato-quadrates and Meckel's cartilage. The most ventral of these rays lies in a single continuous opercular fold which is shown in Powrie 198 and 206 to stretch across between the two rami of the lower jaw and cover the whole ventral region of the pharynx until its hinder border overlaps the anterior ends of the large bones which form the ventral part of the shoulder girdle. This part of the operculum contains irregular rows of very elongated, narrow but thick ornamental bones which lie in festoons parallel to the lower jaw, with the ventral row of whose dermal covering they seem to be continuous.

L. 1209*a*, Manchester, shows the opercular apparatus well. In this specimen it seems that the small displaced rays of B.M.N.H. 38596 are represented by similar structures in their natural position, and that they lie in series dorsally to the large opercular rays which are so well shown in that specimen. Part of the shoulder girdle hides the hyoid arch, but the first and second branchial arches are well shown and agree exactly with those in B.M.N.H. 38596.

It is thus obvious that the most anterior and dorsal part of this operculum is attached to the mandibular arch, and I think it probable that the whole structure I have just described belongs to that cranial segment. But the unfortunate break in the structure in B.M.N.H. 38596 makes it possible to regard the series of large rays, and of course everything lying below them, as of hyoidean position.

The hyoidean arch certainly supports an opercular fold. The upper extremity of the outer face of the hyoidean gill septum is covered by a small patch of polygonal bones, similar to, though smaller than those which pass downward behind the eye to the

dermal covering of the cheek. From this area arises an operculum containing the customary very small bones capped by large and sickle-shaped elements exactly similar to those which mark the anterior and upper end of the mandibular operculum. This small patch of operculum can be traced ventrally to the area covered by the polygonal scales. The exactly similar elements which occur in the same position on the first branchial arch are attached anteriorly to a long, dorso-ventrally directed bony splint which lies in the skin of that gill septum. This condition makes it possible that the small rays in B.M.N.H. 38596, plausibly interpreted as parts of the mandibular operculum, really held a similar position on the hyoid arch. If this were the case then they must have passed downward to a point ventral to the upper border of the palato-quadrate and the mosaic of dermal bones lying over it, and we should have direct evidence that the gill slit between the mandibular and hyoid arches was of full size. I feel it difficult, however, to believe that these long splints could have been stripped away leaving undisturbed a small patch of operculum of much more delicate structure, to which they once gave support. It is thus probable that, in contrast to the first branchial arch, the hyoidean arch remained with a very delicate dermal coating, presumably because it lay for the greater part of its length mesial to the palato-quadrate.

The first branchial arch has its dorsal end covered by a patch of polygonal bones similar to, though smaller than that which occupies the corresponding region of the hyoid arch. Below this the outer surface is covered by a series of narrow, elongated bones in each of which the lower part of the long anterior border rests on the upper part of the posterior border of the one lying below it. The series forms a very narrow belt extending from above downward and a little backward. The whole carries an opercular fold whose dorsal part is supported by slender sickle-shaped bones below which is a region with small ossicles like those in the corresponding regions of the mandibular and hyoid opercula. At the level of the most dorsal of the large mandibular opercular rays one of these splints has a backwardly turned extremity, and from this point downward there is an alternation of free rays and backwardly directed lower ends of splints forming a structure which, though on a much smaller scale, reproduces that attached to the mandible. The whole series then ends abruptly.

The second branchial arch has no dorsal coating of polygonal bones attached to the margin of the gill chamber. It bears a long series of splints which extend downward to a point ventral to the termination of those of the first arch. The lower end of this series turns a little forward. The upper edge of the operculum of this arch has the usual sickle-shaped ossicles, but the series of definite rays begins higher and extends farther down than in the case of the first branchial arch.

The third arch stands more vertically than the second; it has the same structure and its forwardly turned lower end lies even more ventrally.

Finally there is evidence of a short but similar fourth arch.

The general structure of the whole arrangement is obvious. The fish possessed a series of one pre-hyoidean and five branchial gill slits, each of very great dorso-ventral

extent. An independent operculum was attached to the hinder border of each visceral arch from the mandibular to the fourth branchial. The backward extension of the palato-quadrate and Meckel's cartilages, necessary for the formation of the mouth and the attachment of a powerful jaw musculature, brought the hinder end of these elements over the lateral surface of the hyoidean arch and enabled the mandibular operculum to extend backward for a great distance, until in fact its ventral part came to rest on the front margin of the lower part of the shoulder girdle. This operculum, however, left uncovered a large triangular area above its dorsal margin. It is necessary for the functioning of the gill apparatus that the gill slits so left exposed should be provided with an opercular apparatus. This is done by the retention as functional structures of the dorsal parts of the small opercula (the homologues of the flaps in *Scyllium*, which become the frills of *Clamydoselache*) on the hyoidean and branchial arches. Each of these structures is provided with a bony skeleton, which ceases abruptly where it is overlapped by the main mandibular operculum. In fact *Climatius reticulatus* gives us for the first time a stage in the evolution of an operculate fish in which a relic of the Elasmobranch condition of the gill slits is still preserved.

The lateral-line of the body is represented by a groove which runs between two opposed rows of scales. This line continues along the free margin of the scaled area above the gill chamber and extends forward over the gill region to the patch of enlarged bones above and behind the eye. Above the upper ends of the second and third branchial arches short side branches of the lateral-line system are shown in B.M.N.H. 38596 to arise from the main "canal". The remainder of the lateral-line system is very incompletely shown, but it is evident that the arrangement agrees with that perfectly shown in *Climatius uncinatus* and *Euthacanthus macnicoli*. The greater part of the lateral-line system seems to have lain quite superficially above the exoskeleton, but in the anterior part of the main canal and the posterior part at any rate of the supra-orbital canal it lay deeper, the pores connecting it with the surface passing through circular holes made by a coincidence of notches in the borders of neighbouring bones.

Body—The most complete specimen of *Climatius reticulatus* is No. L. 12096, A and B, in the Manchester Museum, in which the anterior end is dorso-ventrally compressed and the hinder part of the body seen in side view, the tail being complete. A restoration made from this specimen shows an unexpectedly slender, fusiform fish, narrower at the pedicel of the caudal fin than most Acanthodians, with a long, very delicate upper caudal lobe directed only very slightly dorsally. No. 202, Powrie Collection, a laterally compressed fish, gives independent confirmation of this restoration. The squamation varies little in its character in different regions of the body; essentially the whole fish is covered with the customary square, Acanthodian scales which become smaller and lose their angles in the lower lobe of the caudal fin. The middle dorsal line of the body between the two dorsal fins bears a narrow strip, about six (?) scales wide,

of rather larger scales, and similar rows lie along the upper and lower margins of the tail pedicel.

Median Fins—The anterior dorsal fin is supported by a spine of enormous antero-posterior length. This spine is laterally compressed, but is in all cases so crushed that its width cannot be determined. The outer surface bears an ornament of sharp-edged continuous ridges which at the base of attachment may number more than twenty, the

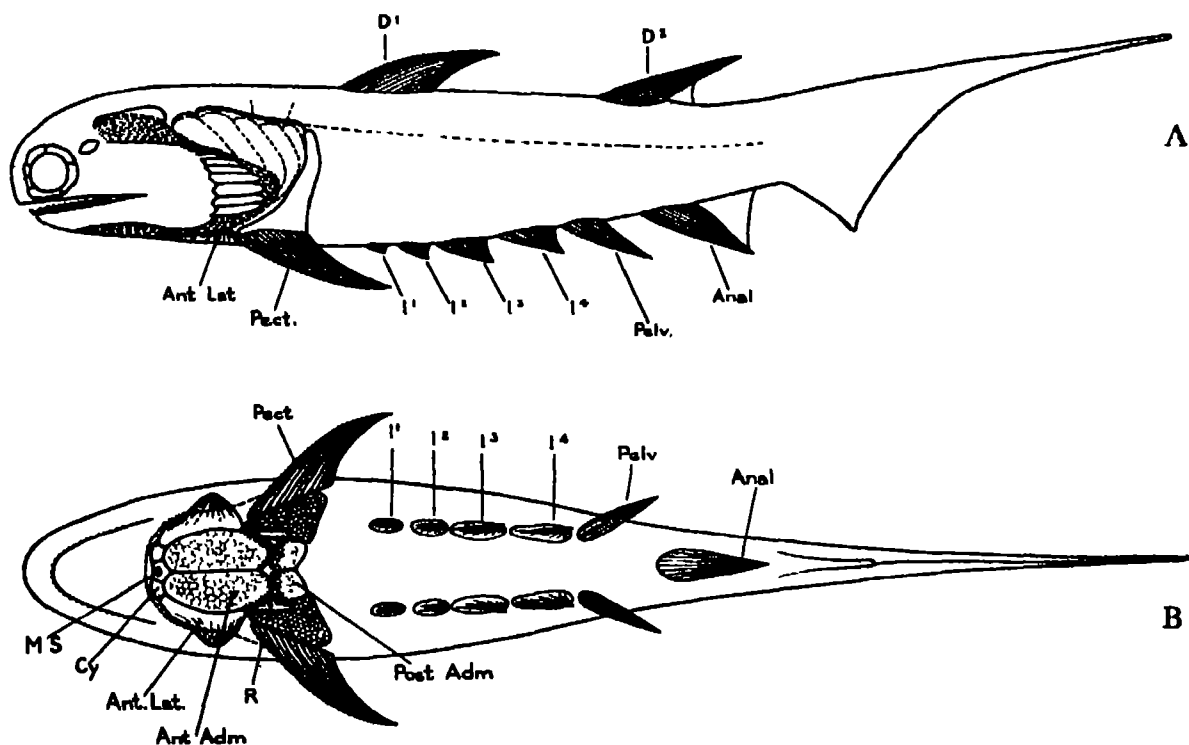


FIG. 2—*Climatius reticulatus* AG. $\times 1.0$. Reconstruction of fish in left lateral view, A; ventral view, B. Anal, anal fin; Ant. Adm. anterior admedian dermal bone of shoulder girdle; Ant. Lat. anterior lateral of shoulder girdle; Cy. cylindrical dermal bone of shoulder girdle; D¹ and D², dorsal fins; I¹–I⁴, intermediate spines; M.S. median dermal bone of shoulder girdle, Pect. pectoral fin spine; Pelv. pelvic fin spine; Post. Adm. posterior admedian of shoulder girdle; R. ridged dermal bone of shoulder girdle.

number being gradually reduced by the termination of individual ridges until toward the tip of the spine only about four or five remain. The spine is inserted very obliquely into the back and has no inserted base whatsoever, so that its attached margin fits into the ordinary squamation of the body, coming in contact with the lateral margins of scales in exactly the same way as each scale touches its neighbours. The basal two-thirds of the spine contain a very large conical cavity surrounded by thin walls; distally the whole becomes "solid". It is uncertain how far, if at all, the cavity of the spine opens posteriorly into the space within the fin membrane, no trace of which is

ever to be seen. The second dorsal and anal fins have exactly the same structures as the first dorsal, except that in them the spine is a narrower structure and stands more vertically. Powrie 1891, 92, 206 shows a series of short ceratotrichia in the base of the anal fin.

The caudal fin includes the very prolonged and slender end of the body, and a small triangular hypocaudal expansion.

Paired Fins—The pectoral fin spines are long, well curved and very wide at their oblique base of insertion. The spine has an anterior ridge which bears a series of low, rounded points, the whole suggesting a series of overlapping scales. The ridges, about twelve in number, ornamenting the flat dorsal and ventral surfaces, bear similar but much lower projections. These spines, like that of the first dorsal which they greatly resemble, are hollow for a great part of their length. The spine is supported by a scapula which is certainly of subdermal origin. It stands vertically following the curve of the posterior end of the gill chamber and can be displaced without affecting at all the squamation lying in the skin over the pectoral region. The scapula rests with its lower border tightly attached to the basal edge of the dorsal surface of the spine, the two structures being so firmly attached to one another that they can be displaced and crushed without dislocation. The ventral surface of this region is covered by a series of powerful dermal bones which are functionally part of the shoulder girdle.

It is evident from the account of the structure of the pharyngeal region given above that the continuous part of the body below the gill slits was narrow and that in front of the pectoral fins there lies a triangular but rounded part of the ventral surface whose margins form the lower borders of the gill chambers. The margin of this area seems to have borne a series of dermal ossicles including anteriorly a low median spine (*M.S.*), to whose lateral border was attached a short, cylindrical bone (*Cy.*). Immediately in front of the pectoral spine there is another bone of a triangular shape forming part of a cylinder resting on the lateral and lower surfaces of the ventral part of the body and extending upward toward the gill slits, so that it is largely concealed by the operculum in lateral view (*Ant.Lat.*). The whole arrangement is completed by a bone, bearing a high, hollow, antero-posteriorly running ridge (*R.*) which is connected to the basal border of the ventral side of the pectoral spine by an area of tightly interlocked small bones, and by a flat dermal one (the anterior admedian) attached to the admedian edges of this bone and that which lies in front of it (the anterior lateral). This element meets its fellow in the middle line. Each anterior admedian lies in front of a small post-admedian which forms part of the posterior border of the shoulder girdle. The whole very complex arrangement can only be made out by careful comparisons of all the existing materials, especially specimens No. B.M.N.H. 38596; Manchester Museum L. 12096 A and B; Edinburgh 1887, 355 and 8a; Powrie 206, 204, 203 and 198. It is clear that there are minor differences between the conditions found in these specimens, but the bones are all recognizable as individuals.

There are usually three pairs of intermediate spines, but four may occur. Each is a mere conical cap attached to the ventro-lateral surface of the body by an oblique base. The outer surface is ornamented distally by longitudinal ribs which break up into points basally. The central cavity extends nearly to the tip of the spine. The distribution of these spines is shown in the restored figures.

The pelvic fin spines are similar to, though shorter than, the anal fin spine, and they have essentially the same structure. No supporting elements are to be seen at their base.

EUTHACANTHUS MACNICOLI

Euthacanthus macnicoli POWRIE. The fish called *Euthacanthus macnicoli* by POWRIE was subsequently referred by SMITH WOODWARD to the genus *Climatius* and is unquestionably nearly related to that animal.

It is found rarely at Turin Hill, so it is fortunate that several specimens, the type Powrie 1891, 92, 231, Edinburgh; its counterpart No. P. 1337, B.M.N.H.; Powrie 236 and another unnumbered specimen in Edinburgh; No. 3329, the Museum of Practical Geology; and P. 295 in my own collection, are very well preserved and between them show all the more important features of the animal.

The fish, which is about 18 cm. in length, has a short rounded head, with rather large orbits placed quite anteriorly; its body is fusiform and apparently nearly circular in cross-section. The tail pedicel is deep and the caudal fin heterocercal. There are two dorsal fins and an anal fin, pairs of pectoral and pelvic fins and a series of five pairs of intermediate fins, all having anterior spines.

The head is similar in general morphology to that of *Climatius reticulatus*, but differs not only in proportions but in many important structural characters. No traces of the neural cranium nor of the endoskeleton of the visceral arches are present, and it is certain that they were entirely unossified. The dorsal surface of the head is covered with the normal body scaling of very small (0.3 mm. square) regularly arranged scales as far forward as an irregular transverse band at about the level of the upper end of the hyoid arch. At this level the scales increase in size a little and become polygonal, losing their very regular arrangement and becoming massed into irregular longitudinal rows.

The main lateral-line of the body passes forward over the gill chamber along the marginal row of scales and then continues along the larger scales which border the dorsal surface of the head to pass mesially of the dorsal orbital bone. The scaling of the head seems to end abruptly at a point above and a little in front of the middle of the orbit, the anterior extremity of the head being naked.

The hinder ends of the supra-orbital canals are clearly shown to pass backward on the inter-orbital surface, each lying between two parallel rows of dermal bones; they end some distance behind the orbit.

In the posterior part of the dorsal head scaling are two pairs of lateral-line grooves;

the posterior arises from the main canal at the point where the body scaling ends and passes inward and forward at about 45 degrees to the middle line. The more anterior one begins in the immediate vicinity of the termination of this groove, and may actually join it. It then passes directly laterally to join the main canal.

The orbit is surrounded by a ring of plates of very delicate structure agreeing in their general character with those of *Climatius* and, like them, being part of the dermal head skeleton. It is unfortunately impossible to determine their detailed arrangement, but

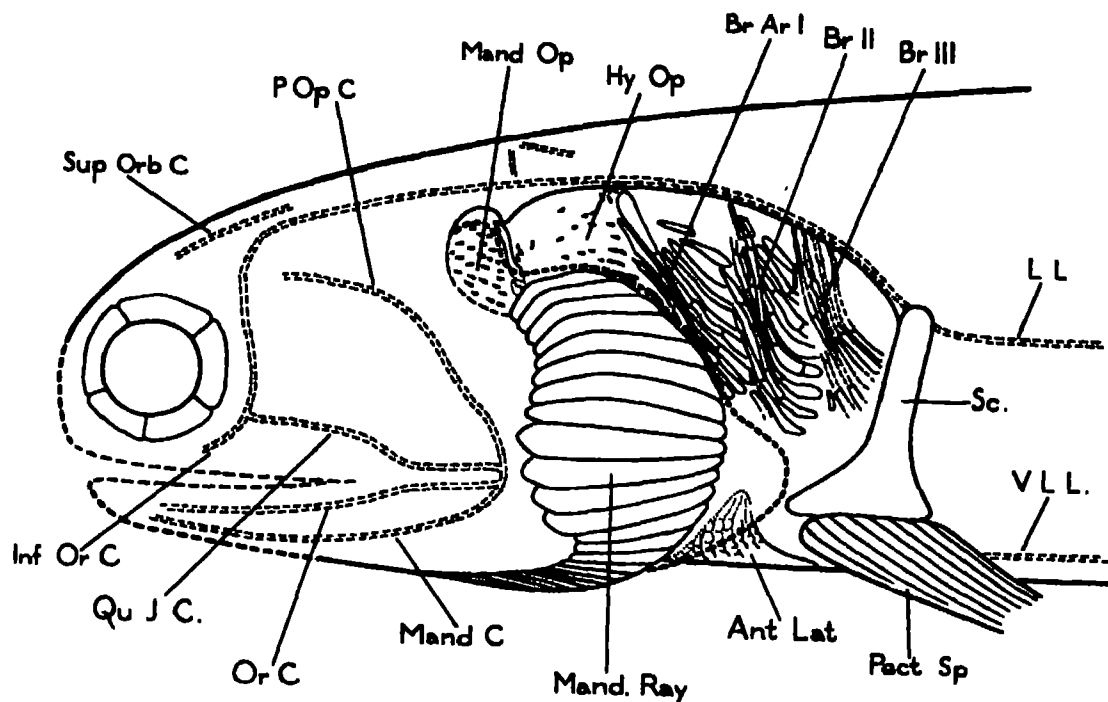


FIG. 3—*Euthacanthus macnicoli* POWRIE. L.O.R.S. Turin Hill. Reconstruction of the head entirely from the type specimen. $\times 3.0$. *Ant.Lat.* antero-lateral bone of shoulder girdle; *Br.Ar. I*, *Br. II*, etc., the dermal bones of the branchial arches; *Hy.Op.* hyoidean operculum; *Inf.Or.C.* infra-orbital canal; *L.L.* lateral-line; *Mand.C.* mandibular canal; *Mand.Op.* upper end of the mandibular operculum; *Mand.Ray*, a ray in the mandibular operculum; *Or.C.* oral canal; *P.Op.C.* preopercular canal; *Pect Sp.* pectoral spine; *Qu.J.C.* quadrato-jugal canal; *Sc.* scapula; *Sup.Orb.C.* supra-orbital canal; *V.L.L.* ventral lateral-line.

it is evident that two lie dorsal to the eye and that there are at least three others, and probably more.

Behind the orbit lies the cheek whose bony covering is connected with that of the dorsal surface of the head by a patch of unusually large bones, forming a narrow neck in front of the spiracular cleft, over which passes the upper end of the infra-orbital lateral-line canal. This lies considerably behind the orbital ring of bones and can be traced downward between two regular rows of very small bones nearly to the border of the mouth. Behind this groove the cheek passes upward, without any sudden break in

its squamation, into the mandibular operculum which covers the upper end of the spiracular gill slit. This structure is covered toward its free margin by exceedingly small and delicate scales. Posteriorly the cheek contains a belt of relatively very large bones mapping out the course of the pre-opercular canal from its origin from the upper end of the infra-orbital canal to its termination at the articulation of the lower jaw. A lateral-line groove, the quadrato-jugal canal, passes forward from the lower end of the pre-opercular canal to join the infra-orbital canal at its lower end. Behind the canal the cheek extends backward with its normal scaling until it passes abruptly into the main part of the mandibular operculum.

The lower jaw is toothless and is represented solely by the two rows of small narrow bones which support the lateral-line grooves, and by an excessively delicate squamation of very small isolated scales. There are two lateral-lines, one near the ventral border of the jaw, the mandibular canal, the other, the oral canal, which is shorter, joining the main canal posteriorly and lying near to the probable dorsal margin of the jaw; both arise from the lower end of the pre-opercular canal.

The first operculum, which on the evidence of the conditions obtaining in *Climatius reticulatus*, *Brachyacanthus scutiger* and later Acanthodians is presumably of mandibular arch origin, is supported by a very extensive series of twenty-five powerful, elongated bones. This series extends from a point about on the level of the horizontal part of the pre-opercular canal to one which must lie toward the middle line between the lower jaws. The most dorsal element has its anterior part directed upward and backward from its attached extremity, it then turns horizontally backward. The five rods immediately below also retain their downturned attached ends, but their distal parts widen rapidly so that by the eighth the whole bone is sensibly straight. The ninth to twelfth rods have wide attached ends and narrow distally, the anterior ends gradually turning upward below the level of the articulation of the jaw. This fan-shaped arrangement is extremely characteristic and is preserved in such more advanced forms as *Mesacanthus*, *Cheiracanthus* and *Acanthodes* itself. The thirteenth rod is narrow from top to bottom but of considerable length. The remaining twelve are slender and shorten gradually. The type specimen shows a small series of fine, slender long rods, lying out of series with the elements of the ventral ends of the right and left opercular folds. These presumably represent the skeleton of the median part of the continuous opercular fold, and parallels to them can be found in *Mesacanthus* and *Acanthodes*.

The hyoidean arch is shown on each side of the type specimen. There is only a very slight indication of a scale-covered area on the upper end of the gill septum; this is followed directly by two long, slender ossicles which stand vertically, the lower lying in front of the more dorsal. The ventral end of the upper bone is turned backward, and the lower bone in the specimen comes into contact with the upper bone of the large mandibular opercular rays, the direction of the two bones being totally different. The hyoidean operculum is seen as a structure supported by delicate oat-shaped ossicles lying horizontally, well separated from one another. The free margin contains a single

series of large bones which reach, and presumably rested on, the first branchial arch.

The first branchial arch is similar in its general structure to that of *Climatius*. The outer surface of the narrow gill septum is covered by a series of fine, long, slender rods, similar to the two at the summit of the hyoid arch. Each of these is turned backward horizontally at its widened lower extremity, and additional rays of exactly the same character are inserted between some of these backturned ends, so that there are actually eight visible. The whole structure ends abruptly at the level of the articulation of the upper and lower jaws, that is, the level of the longest mandibular opercular ray.

The second branchial arch has the same structure as the first, but seems to possess six rods and eight rays and to extend to a slightly more ventral point.

The third branchial arch is again similar, but the rods are more sharply bent where they turn backward as rays, and the structure is hidden by scales before its natural end. No trace of any fourth arch is visible.

It will be noticed that the abrupt ventral termination of all the ossifications in the hyoid and branchial arches, which themselves evidently extended downward toward the mid-ventral line, can only be explained by the presence of a large posterior extension ventrally of the mandibular operculum.

The body as a whole is covered continuously with normal, square Acanthodian scales. These are uniform in size and arrangement, the only noticeable variation being the presence of a strip of rather larger scales along the mid-dorsal line, which with a similar ventral strip forms a stiffening to the caudal pedicel. There is also a patch of similar scales at the attachment of the pectoral fin spines.

The anterior dorsal fin spine is straight and sharp pointed with a very oblique insertion on the body. It has a large cavity extending nearly to the tip and no inserted region, the base lying in the squamation. The spine is ornamented by a series of ridges and deep grooves, none of which bears denticles, nor does the posterior margin. A brown stain in the type specimen may represent the fin web, which has no visible scaling.

The second dorsal fin spine is similar to the first, but is longer and inserted at a more open angle. The web of the fin is covered with square scales of exceedingly minute size, which distally are arranged in rows.

The caudal fin is of great interest; it is typically heterocercal, the hypocaudal lobe being, however, small and long based so that the hinder margin of the whole fin is nearly straight. The strip of enlarged scales along the dorsal surface of the deep pedicel ceases abruptly about a third of the length of the fin in front of the posterior end. It here forms a definite free projection behind which the upper border of the tail is covered by minute scales, the continuation of a strip which extends forward for some distance. Ventrally these pass into the pointed hinder end of the body covered with the normal squamation. The hypocaudal lobe has a thickened antero-ventral margin and is otherwise covered with small scales arranged in rows.

The pectoral fins are remarkable in that only the scapula, and that triangular element which contributes to the lateral surface of the ventral part of the body below the gill slits, are present as large bones in addition to the spine. The web of the pectoral fin is often visible as an area covered with very small square scales. The intermediate pairs of spines form a close-packed series beginning a little behind the pectoral fin and ending the length of a spine in front of the pelvics. There are usually five pairs, but the type specimen has an additional spine at the anterior end on one

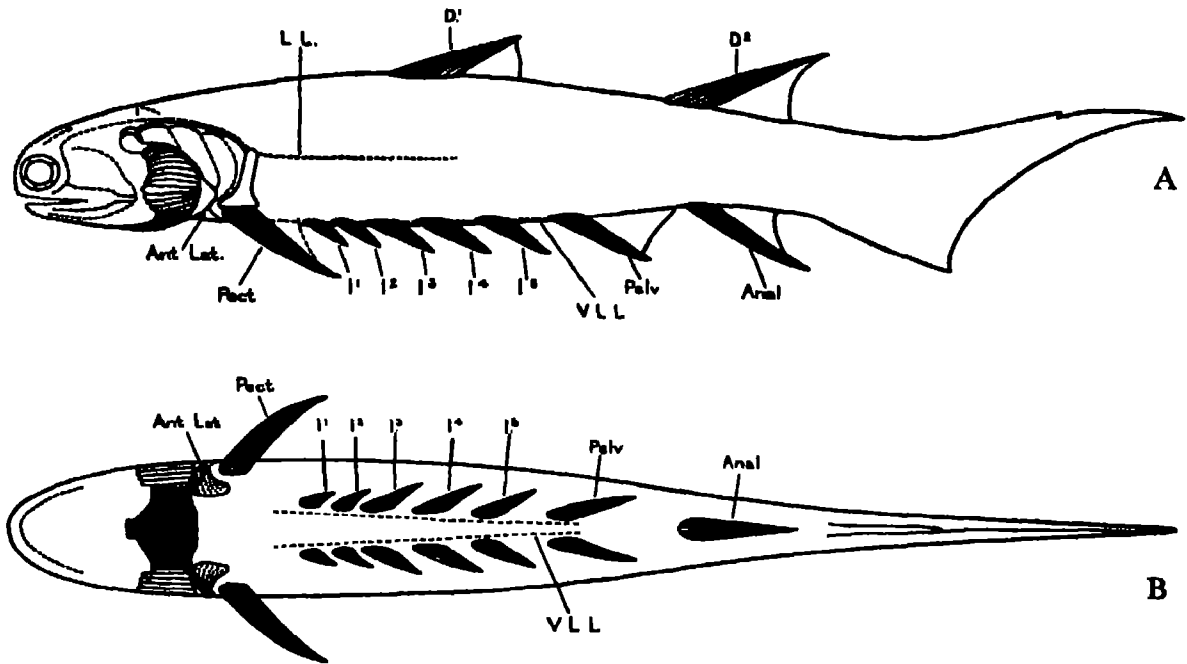


FIG. 4—*Euthacanthus macnicoli* POWRIE. A, lateral and B, ventral reconstructions. $\times 0.9$. Anal, anal fin; Ant. Lat. antero-lateral pectoral dermal bone; D^1 and D^2 , the dorsal fins; I^1 – I^5 , the intermediate paired spines; L.L. lateral-line; Pect. pectoral fin; Pelv. pelvic fin; V.L.L. ventral lateral-line.

side only. These spines have exactly the same structure as the normal fin spines. The series increases in size from first to last, the posterior spine being only very slightly shorter than those of the pelvic fins. I have never seen any trace of fin web, probably because the spines overlap one another closely and never stand out freely beyond the body, the only condition under which the web is ever to be seen.

BRACHYACANTHUS SCUTIGER EG.

The fish described as *Brachyacanthus scutiger* by EGERTON was soon referred by him to *Climatius* AGASSIZ, and has since remained in that genus. It is, however, though a close relation, so different structurally from *C. reticulatus* that it is necessary to revive its original generic name.

Brachyacanthus seems to have been a common fish at Farnell, and has also occurred, though rarely, at Tealing and Duntrune near Dundee; it is doubtful if it really occurs at Turin Hill. It is small, about 7 cm. in maximum length, and appears to have been less slender than *Climatius reticulatus*, the anterior dorsal fin spine being inserted on the summit of an arched back. The tail is long and very slender. All the fin spines are short and exceedingly broad at their bases.

The more important specimens on which the following account is based are: Powrie 1891, 92, 220, from Farnell. This is a mould of the external surface of the left side of the head and anterior part of the body of a large fish. It shows very perfectly the structure of the pharynx, but is at first difficult to interpret, especially in photographs, because fragments of black bone remain in the centre of each individual bone impression and at first appear to be sutures between the individual elements of the skeleton. Powrie 1891, 92, 213, from Tealing, shows the inner surface of the bones covering the top of the head very well. Powrie 214 is a nearly complete fish in lateral aspect, important because it shows from their inner surfaces the large bones of the mandibular operculum. B.M.N.H. P. 9596, which is said to be from Turin Hill, shows well the inner surface of the upper part of the head and, misplaced, the lower jaws and intergular part of the operculum and the check and opercular rays of one side.

The Head—There is no evidence of any calcification in the neural cranium, but the palato-quadrate and Meckel's cartilages may be well "ossified"; they are, however, never sufficiently well exposed to be described. There is no evidence of any calcification in other parts of the visceral arches.

Dorsal Surface of the Head—*Brachyacanthus* differs from all other Acanthodians in the presence of a series of large hexagonal median scutes forming a continuous series from the first dorsal fin spine to the back of the head. The normal squamation of very small square scales usually passes into this median series through the intervention of small, rather irregular scutes which form a vague belt on each side. The most anterior and largest of these median bones lies on the level of the first branchial arch; it may be regarded as the hinder end of the dermal skull roof. From its lateral borders series of small bones pass out laterally forming a transition region between the scaling of the body and those large bones which cover the head. The head plating is irregular, varying not only between individuals but also between sides of the same specimen. The median series may be broken occasionally by pairs of bones. A lateral series of bones with a deeply grooved lower surface is evidently related to the main lateral-line canal and can always be traced. This series is separated from the border of the gill chamber by another series which ends in a patch of bones behind the circlet surrounding the eye. One bone of the patch is always recognizable because its inner surface has a deep rounded pit, and its outer surface is raised into a low pyramidal point. The orbital margin is formed by a series of five large ornamented bones of massive construction. The space between the orbits is covered by two pairs of long bones, the anterior end

of the head being undescribable. The ventral orbital bone appears to form part of the margin of the mouth, which posteriorly lies on a chain of three large bones. These form the lower part of the covering of the cheek which ends dorsally and posteriorly in a continuous chain of bones, associated with the "hyomandibular" lateral line canal, the rest of its area being covered by an irregular mosaic of polygonal bones extending

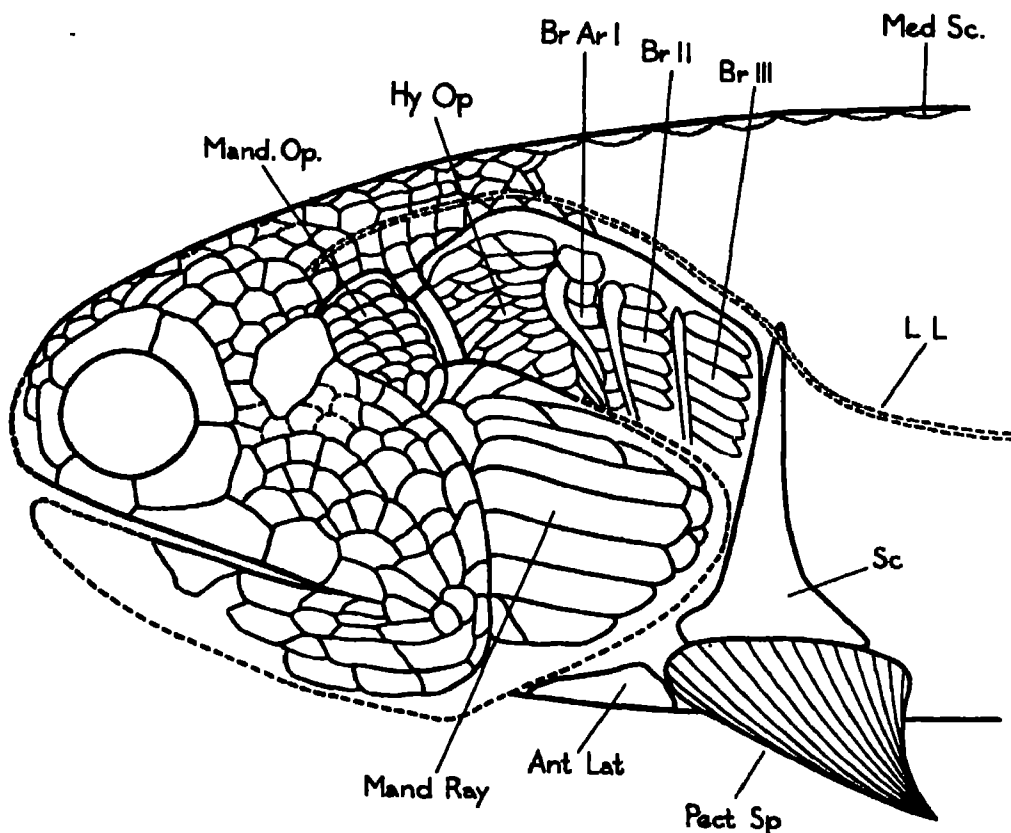


FIG. 5—Head of *Brachyacanthus scutiger* Eg. $\times 6.0$. Lower Old Red Sandstone, Farnell. Reconstructed mainly from specimen, Powrie 1891, 92, 220, Royal Scottish Museum. *Ant. Lat.* antero-lateral dermal bone of the shoulder girdle; *Br Ar. I*, *Br. II*, etc., the branchial arches and their opercula; *Hy. Op.* hyoidean operculum; *L. L.* lateral-line; *Mand. Op.* the upper part of the mandibular operculum; *Mand. Ray*, one of the rays of the mandibular operculum; *Med. Sc.* median scute; *Pect. Sp.* spine of the pectoral fin; *Sc.* scapula.

forward to the circum-orbital series. The posterior part of the lower jaw is covered externally by four irregular longitudinal rows of bones.

The mandibular operculum agrees in principle, and even largely in detail, with that of *Climatius reticulatus*. The small anterior portion which lies immediately in front of the upper portion of the hyoid arch, on which its posterior border rests, is supported by horizontally arranged rows of small bones. These extend down to the upper border of the cheek plating whence the main portion of the operculum begins with a radiating

series of relatively large bones whose lower borders eventually become horizontal and rest on the uppermost of the series of five long rays. These rays articulate with the hinder border of the cheek and extend backward to join an irregular series of polygonal bones which form the actual hinder border of the operculum, representing a group of very small ossicles in a homologous position in *Climatius*. The specimen Powrie 220 is of very great importance, because in it the upper border of the mandibular operculum as it crosses the hyoidean operculum is clearly defined, and it is quite obviously an independent and more superficial structure. Specimen P. 9596 shows the whole series of opercular rays of the left side in position behind the cheek, the lower jaws retaining their original position with respect to these structures, the whole being seen partly as an impression from within. The transition from the lateral part of the operculum, through a region which is very short because it is placed between the lower jaw and the shoulder girdle, to the large gular part of the fold which is continuous and free across the middle line, is very well shown in this specimen. The gular part of the operculum, as in *Climatius*, is beset with longitudinally arranged rows of narrow but elongated bones.

The upper end of the hyoidean arch is covered by two bones, articulated with the head plates and projecting beyond the border of the gill chamber, exactly as does the corresponding structure in *Climatius*. Below these the lateral surface of the gill septum is covered by what is apparently a single vertically placed element whose lower end is covered by the mandibular operculum. The hyoidean operculum is an extensive structure with free upper and posterior margins. It contains ten horizontal rows of shallow elongated bones.

The outer surface of the first branchial arch contains, towards its upper end but not in contact with the margin of the gill chamber, a single isodiametric bone below which lie three narrow splints each overlapping the one below and in front of it. In the actual specimen this arch lies very near the second branchial, and its operculum is in consequence not very clearly shown.

The second and third arches have only a single long splint, and each supports a short deep operculum containing horizontal rays which seem to be continuous throughout their length.

The whole structure of the branchial region in *Brachyacanthus* thus agrees with that in *Climatius*, differing only in the general proportions and especially in the fact that all the "bones" in the various opercular folds are very much bigger and more massive. It is of importance because it gives independent and very satisfactory evidence that the first and largest operculum is actually a structure arising from the mandibular arch and is quite independent of the hyoid.

Median Fins—The first dorsal fin spine arises from the most dorsal part of the body. It has a very widely expanded base whose border meets the last of the median scutes in front, and for the rest of its extent is directly in contact with the ordinary squamation.

The whole structure contains a very large cavity which extends nearly to the tip, the wall of the spine being exceedingly thin. In profile it is evident that both the anterior and posterior surfaces are concave, so that the tip is sharply pointed whilst the base is enormous, actually as long as the height of the spine. The lateral surface of the spine is ridged, the number of ridges at the base being about twelve, of which some three only reach the tip, the others dying out. The fin web is often preserved. It is covered with very small quadrangular scales without a very definite arrangement.

The second dorsal spine is longer and more slender than the first, but is otherwise similar. The fin itself differs in that its scales are very regularly arranged in rows which radiate out like the ribs of a fan from an area covered with less regularly arranged scales at the base of the posterior edge of the spine.

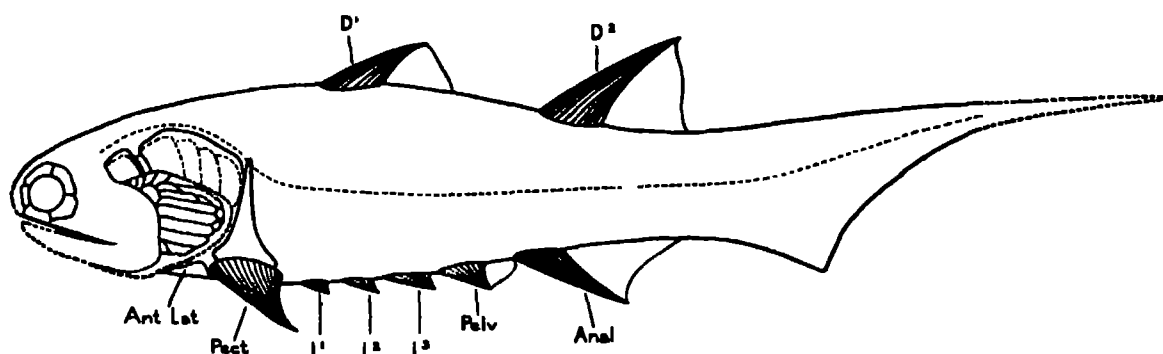


FIG. 6—*Brachyacanthus scutiger* EG. $\times 1.8$. Reconstruction of the fish from material from the Lower Old Red Sandstone of Farnell and Duntrune. *Anal*, the anal fin; *Ant. Lat.* antero-lateral dermal bone of the shoulder girdle; *D¹* and *D²*, the dorsal fin spines; *I¹*, *I²*, *I³*, the intermediate paired fin spines; *Pect.* pectoral fin; *Pelv.* pelvic fin.

The caudal fin was well figured by EGERTON; it is remarkable for the extreme length of the upper lobe, perhaps even exceeding the condition in *Climatius*.

The anal fin spine is very like that of the second dorsal, but the fin itself differs in that the rows of scales on it lie parallel to one another and end on the body and not at the point of insertion of the spine.

The pectoral girdle is not well displayed in any specimen, and I am uncertain of its structure. The scapula is always visible. It differs considerably from that of any other Acanthodian because its shaft is a narrow rod ending dorsally in a point and widening abruptly at its lower extremity to form a hollow plate-like structure which is in some way attached to the margin of the widely open base of the spine. The spine is a hollow conical cap of dentine with very thin walls; its attached margin is oval, nearly as deep dorso-ventrally as it is long, and the spine from base to point along its posterior margin is no longer than the length of its attachment to the body. The outer surface bears a series of longitudinal ribs which proximally bear low points in regular series. There are about twelve rows of ribs on each surface at the base and three distally.

There appears to be one pair of large bones on the ventral surface between the pectoral spines and the hinder border of the operculum.

The three pairs of intermediate spines and the pelvics form a close-set series, each almost in contact with its neighbours. The most anterior, which lies about its own length behind the pectoral spine, is little more than a large oval scale with a horizontal hollow rib having a free extremity. The pelvic spine differs only in being much larger, the attached base being about twice as long, though of nearly the same width, and in having a much more pronounced rib with a definite spine posteriorly. The intermediate spines are exactly intermediate in structure.

It is important that specimen Powrie 214 shows a well-preserved pelvic fin web extending back from this spine to that of the anal fin. In Powrie 213 an enlarged scale lies immediately in front of the first intermediate spine, resembling it in outline but lacking any trace of rib or spine.

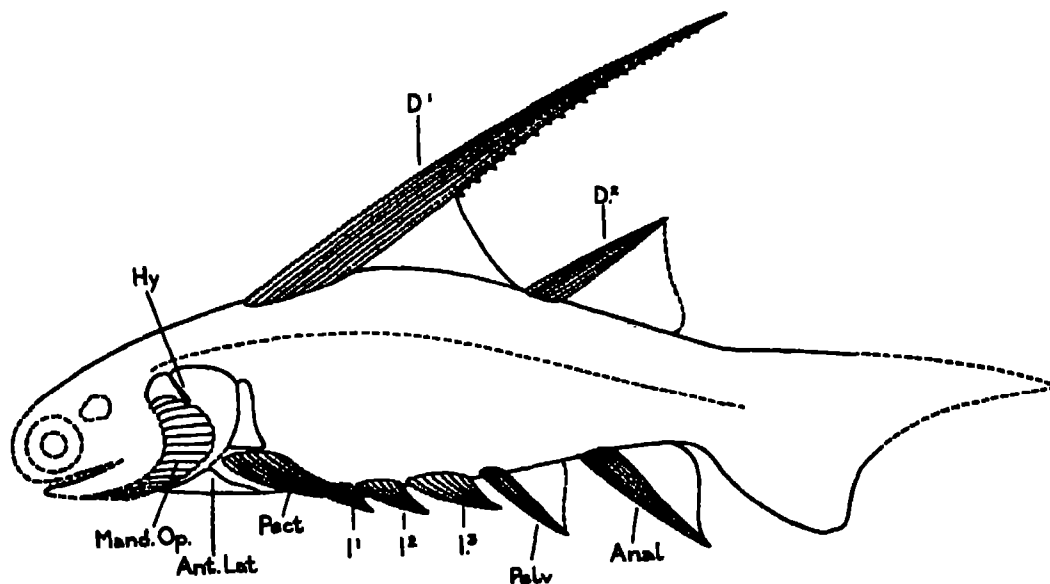


FIG. 7—*Parexus incurvus* AG. $\times 1.2$. Restoration of the fish from specimen L. 12097 B, Manchester Museum. *Anal*, anal fin; *Ant.Lat.* antero-lateral dermal bone of shoulder girdle; *D¹* and *D²*, dorsal fins; *Hy.* upper end of the hyoid arch; *I¹*, *I²*, *I³*, the intermediate paired spines; *Mand.Op.* the mandibular operculum; *Pect.* pectoral fin; *Pelv.* pelvic fin.

PAREXUS INCURVUS AG.

Agassiz, L. 1845.

Powrie, J. 1864, pl. XX, fig. 1.

Powrie, J. 1870, pl. XII, fig. 8.

Woodward, A. S. 1891, fig. 4.

Parexus incurvus and its larger ally *P. falcatus* are rare fish at Turin Hill, and the material available does not allow me to give so complete a description of either as of the other species dealt with in this paper.

In fig. 7 I give a reconstruction founded on the specimen L. 12097 A and B, Manchester Museum. This shows the extraordinary build of the fish which depends on the exaggerated spine of the first dorsal fin.

The head agrees in its general structure with that of *Climatius*, having a very similar dermal skull roof and cheek, but the mandibular operculum, like that of *Euthacanthus*, contains a long continuous series of rays. The hyoid and branchial arches are very similar to those of *Euthacanthus*.

The dermal shoulder girdle is well developed but contains only a single pair of very large bones on the ventral surface, described by SMITH WOODWARD as clavicles.

The first dorsal spine is peculiar in possessing two rows of distally directed denticles on its posterior surface, the denticles on the right and left sides of the spine alternating regularly.

The lower jaw has a dentition resembling that of *Climatius* but in which the individual teeth have narrower cusps.

The head mentioned by TRAQUAIR, and described by SMITH WOODWARD (1915) as *Protodus scoticus*, is clearly that of an Acanthodian, and in all probability of *Parexus*.

MESACANTHUS MITCHELLI

Mesacanthus mitchelli is found abundantly at Turin Hill and Farnell in the well-known, very finely bedded calcareous and micaceous shales which there form rich fish beds. It has also been found very rarely as isolated individuals in small pockets of shale included in the sandstones at Carmylie and Ley's Mill in Forfarshire, all these localities being very nearly the same horizon in the lower Old Red Sandstone. Individuals are usually preserved in exact profile, but two of my series of fifteen were dorso-ventrally compressed, so far as their anterior extremity is concerned. It therefore appears that the fish, though in the main laterally compressed, had a head which may have been nearly circular in section. It is clear that the snout was rounded and blunt, and the relatively very large orbit lies so far forward as to show that the olfactory organs must have been small. The mouth is a little underhung, the lower jaw ending under the anterior border of the orbit and not extending quite so far as the extremity of the snout. The head is small, about two-elevenths of the total length. The fish as a whole is well stream-lined, though the root of the tail is deep, about half the maximum total depth of the fish in the region of the pectoral fins. The caudal fin is clearly heterocercal, but its upper lobe is only very slightly upturned. The hypocaudal lobe is small and triangular but with a short and stiff recurved point. The two unpaired fins lie with the dorsal a little behind the anal, which is almost accurately in the middle of the length of the fish. The dorsal fin spine is about four-fifths of the maximum depth of the body, the anal spine is slightly shorter, each is inserted at an angle of about 45° to the body outline. The dorsal spine is ribbed, or rather grooved, there being one rather deeper and wider groove extending the full length of the spine and smaller

but parallel grooves, some of which do not continue to the tip. The web of the fin is only very rarely indicated by slight traces of scales which do not enable one to decide how nearly it extended to the tip of the spine.

The paired fins are three in number, each supported by an anterior spine similar in its general character to those of the dorsal and anal fins, but apparently asymmetrical. The pectoral fin spine is of about the same length as the dorsal but is considerably stouter; its outer dorsal surface bears an irregular and rather shallow groove; its inner ventral surface, on the other hand, has an anterior deep and wide furrow extending the whole of its length, and bears two or three much less well-defined grooves only on its basal half. No trace of the fin membrane is preserved.

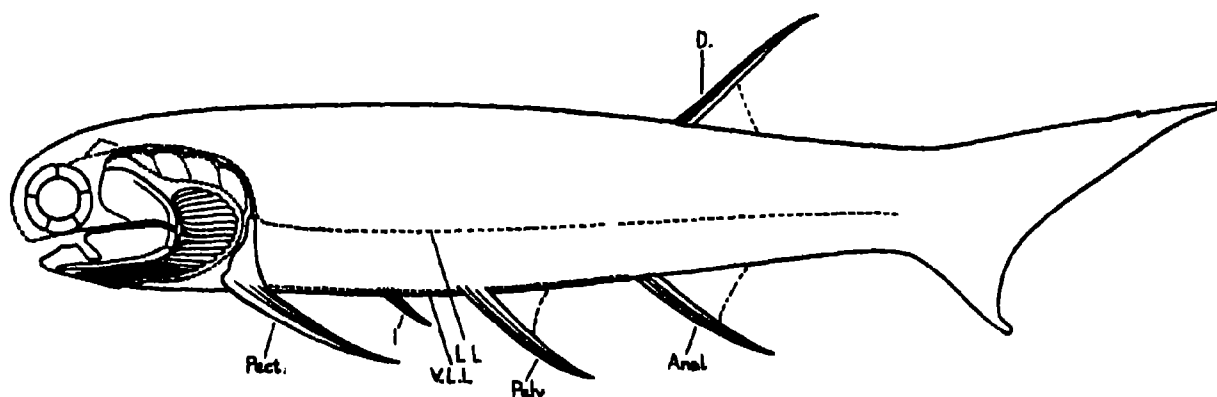


FIG. 8—*Mesacanthus mitchelli* EG. $\times 3.0$. Reconstruction of the fish founded on material from the Lower Old Red Sandstone of Turin Hill. Anal, anal fin; D. dorsal fin; I. intermediate pair of spines; L.L. lateral-line; Pect. pectoral fin; Pelv. pelvic fin; V.L.L. ventral lateral-line.

The intermediate fin spines are short, rather broad, and to some extent grooved. They lie close together on the ventral surface of the fish, seemingly parallel to the principal plane.

The pelvic fin spines are relatively more slender and much longer, being indeed about two-thirds the length of the pectoral spines. Each of them, apparently on its lateral surface, has one deep continuous groove behind which lie several much smaller ridges and furrows forming little more than a striation. Slight traces of the web of these fins can be seen.

The fin spines of *Mesacanthus* are purely superficial structures inserted entirely into the skin amongst the scales, and not penetrating into the body between muscles as do those of Elasmobranchs and later Acanthodians. The individual spine has a cavity which extends nearly to its tip and communicates by a wide opening with the subdermal tissues of the animal. The bone which forms the border of this opening lies on the same level as the body scales, whose free extremities may overlap the base of the spine.

The greater part of the external surface of the fish is covered with a very close-fitting squamation composed of extraordinarily small scales. The scales measure about one-

sixteenth of a millimetre across, are almost accurately square, and overlap from before backwards. They are disposed in rows running obliquely at an angle of 45° to the length of the fish, the rows cutting one another at right angles. This very regular squamation alters its character a little in certain regions. The lateral-lines are clearly marked by a slight local enlargement of the scales, leading to a disorganization of their very regular arrangement. Laterally, in the region between the pectoral and pelvic fins, the scales become markedly larger and less square-cut in outline, and the base of the pelvic fin spines is surrounded by somewhat rounded scales, some of which are three times as big as those which cover the flank. Similar enlarged scales occur in front of the dorsal and anal fin spines, but behind these the marginal scales of the body are exceptionally small and lose their regular tessellated pavement arrangement.

The upper lobe of the tail is covered with scales which tend to become elongated, overlapping one another more deeply than those of the trunk; they suggest indeed the rhomboidal scales which occur in this position in Osteolepid fishes. The mid-dorsal line of the caudal fin is specially protected by a series of particularly enlarged scales, apparently paired, which overlap one another and form a definitely thickened margin. This suddenly stops at a point about a quarter of the whole length in front of the hinder end of the fin, forming in three specimens a point of marked break in its outline, indeed almost a small definite lobe. The hypocaudal lobe of the caudal fin has its anterior margin protected by a similar row of enlarged scales which continue on behind the termination of its web to form a curious little recurved point. The scaling of the web of this part of the caudal fin is deeply overlapping, and much less regular and massive than that of the upper lobe. In adult specimens, e.g. P. 472, the squamation is as I have just described it, but in young individuals (Powrie 1891, 92, 275) there is a triangular area of the trunk which is free from scales and lies below the main lateral-line and dorsally to the ventral lateral-line over the shoulder girdle. As the fish grows older this region is gradually reduced in size by addition of scales to its dorsal, and perhaps to its ventral margin.

No trace of any ossification in the neural cranium is visible but the specimen Powrie 1891, 92, 275 retains three small dense rounded calcareous elements lying just behind the orbit at a level which must be nearly that of the basis cranii. These can be seen, less well, in other specimens. I am indebted to Miss TOWNEND for the obviously correct interpretation of these very puzzling structures as otoliths.

The only parts of the endoskeletal structures visible in the head of *Mesacanthus* are Meckel's cartilage and the palato-quadrate cartilage. These are displayed in very many specimens as calcified elements, clearly not of the nature of ordinary calcified cartilage. I propose to refer to them as bone, and shall at a later stage of this paper give some justification for using the term. The palato-quadrate bone recalls very vividly that of *Notidanus*. It has a short and slender suborbital part and a very deep post-orbital region rising to a high otic process, which cannot be shown to have touched the neural cranium and in any case presents no evidence of an articular facet.

There is some evidence that the palato-quadrate bone actually consists of a short anterior suborbital bone and a much longer posterior portion. The anterior bone agrees in its general character with that of *Acanthodes*, having in its admesial border a deep rounded notch whose anterior edge forms a facet for articulation with the basis cranii, behind the orbit. The lower jaw bone equally recalls Meckel's cartilage of the

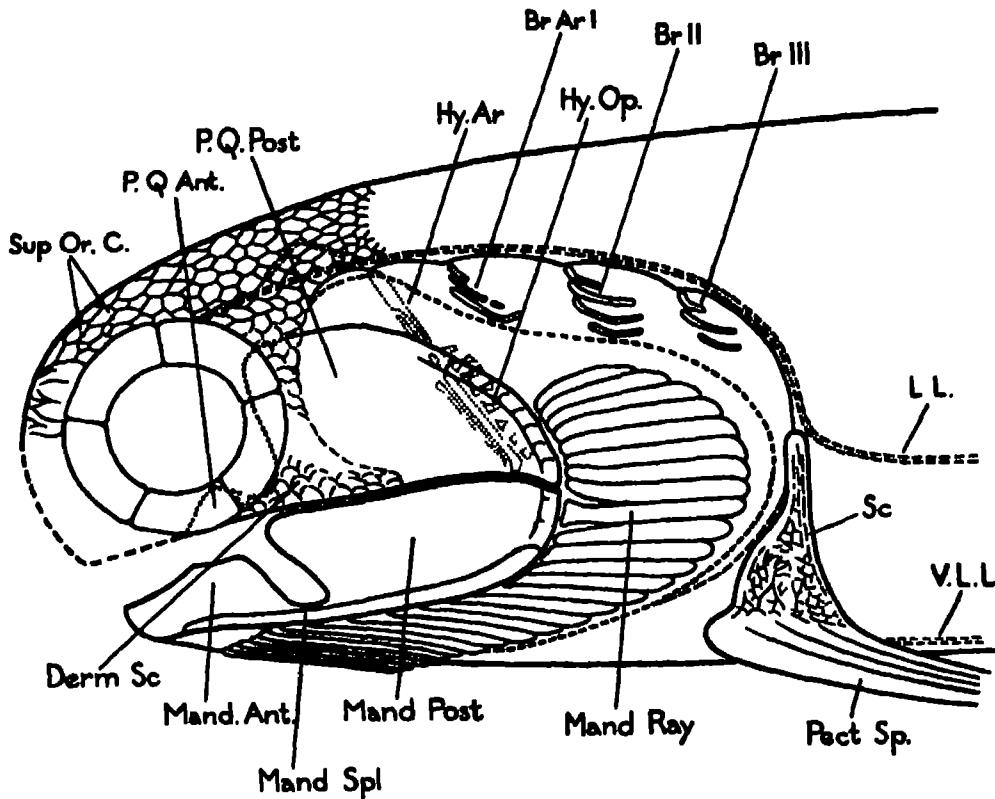


FIG. 9—*Mesacanthus mitchelli* Eg. Reconstruction of head. $\times 9.0$. The hyoid and branchial arches mainly from Powrie, 1891, 92, 275, Royal Scottish Museum. *Br. Ar. I*, *Br. II* and *Br. III*, the dermal bones of the branchial arches; *Derm. Sc.* dermal bones of the cheek; *Hy. Ar.* upper end of the hyoid arch; *Hy. Op.* the hyoidean operculum; *L.L.* lateral-line; *Mand. Ant.* the anterior ossification in Meckel's cartilage; *Mand. Post.* posterior bone in Meckel's cartilage; *Mand. Ray*, a ray in the mandibular operculum; *Mand. Spl.* the mandibular splint; *P.Q. Ant.*, *P.Q. Post.* the anterior and posterior bones in the palato-quadrate cartilage; *Pect. Sp.* pectoral fin spine; *Sc.* scapula; *Sup. Or. C.* supra orbital canal; *V.L.L.* ventral lateral-line.

dogfish; it is shallow anteriorly, deep posteriorly, and bears a concavity which articulates with the posterior and lower corner of the palato-quadrate bone. Neither element bears the slightest trace of teeth. Like the palato-quadrate, the ossified lower jaw consists of two elements, anterior and posterior, which in young individuals are widely separated but fuse completely later in life. A long "bony" splint articulates with the lower border of the jaw throughout its length.

The exoskeleton of the head is very well shown in my series of specimens. The whole dorsal surface, from the anterior end to the region of the gills, is covered with a series of relatively large irregular scales, which, from a point about a quarter of its diameter behind the orbit, pass backwards gradually into the normal squamation of the dorsal surface of the trunk. These scales tend to be longer than they are wide and fall somewhat irregularly into fifteen longitudinal rows. More anteriorly, forming indeed the blunt anterior end of the head, the scales are transversely widened, about four or five covering the inter-orbital space; these may fuse into large plates. The elements which surround the nostrils are sometimes recognizable by their concave rounded borders. It is evident that the nostrils lay on the rounded anterior end of the head between the supra-orbital lateral lines and some way above the border of the mouth. The elements which build up this shield are not rigidly fixed and the whole region has some flexibility, though it is coherent and may be displaced as a whole. At the sides the dorsal shield passes abruptly into the two dorsal elements of the ring of five surrounding the orbit. Their mutual relations are such as to show that these plates, which it is natural to regard as ossifications of the sclerotic capsule of the eye, are actually external dermal elements of the head armour, a view which is confirmed by the ornamentation of the external surface of at least the more dorsally placed elements. The complete series builds up a rather irregular ring, wider dorsally than ventrally. Behind and below the orbit there is little trace of exoskeleton, the greater part of the palato-quadrates and Meckel's cartilage ossifications being visible. A small triangular area, dorsal and posterior to the orbit, is covered with a series of rather large thick scales in five or six irregular horizontal rows, and the mouth below the hinder part of the orbit seems to be bordered by a large ornamented ossification perhaps associated with smaller scales.

The dorsal surface of the head displays the course of the lateral-line canals with clearness. They are visible because of the small perforations, usually lying between two scales, by which their tubuli came out to the surface. They form four parallel series: an inner pair which begins in close contact with the anterior border of the orbit and extends straight backwards along a more or less continuous line of scales for some distance, and another pair which begins above the centre of the orbit, gives origin apparently to an infra-orbital branch, and then passes backward through a continuous line of larger scales to the region of the pectoral girdle and on into the main canal on the body of the fish. In addition the dermal bones of the roof of the head show short lateral-line canals arranged in a V over each ear region. The line of large scales is the most ventral trace of exoskeleton in the body in the gill region, and with the ventral part of the shoulder girdle and a series of enlarged scales extending forward on the ventral surface in front of the pectoral fins, marks out the margins of a well-defined gill chamber.

The only remaining scales in the head are the series of long, powerful rays which clearly have the structure of scales and lie in an opercular fold, so that in the living

fish their free posterior extremities must have rested on the ventral portion of the margins of the gill chamber described above. The arrangement of the opercular bones is very remarkable. On the ventral surface between the lower jaws they lie parallel to one another and to the middle line, the complete series being at least sixteen in number. They do not here overlap the lower jaw, with which indeed they lie parallel, nor do they appear to be related in any intelligible way to the hyoid arch. They are quite long so that the posterior margin formed by the entire series is a wide V-shaped notch, it being impossible to say whether the opercular fold was continuous across the mid-ventral line or not. Posteriorly these opercular rays pass farther and farther backwards until they come to rest on the posterior margin and indeed apparently on the outer surface of Meckel's cartilage and the palato-quadrates. Individually they terminate behind in rather blunt points. The entire series makes a rounded posterior edge to the operculum, which extends backward to the shoulder girdle but leaves the upper part of the gill chamber freely visible. The opercular rays just described are obviously the homologues of those in *Euthacanthus*, and indeed resemble them even in detail. For example, the arrangement designed to secure dorso-lateral flexibility at the point of articulation of the jaw, whereby the attached ends of the rays overlapping Meckel's cartilage are directed downward whilst those on the palato-quadrates are upwardly directed, is the same in the two fish. I have shown reasons for believing that this operculum is part of the mandibular arch and that there was a full-sized normally functional hyoidean gill slit. One specimen of *Mesacanthus* (Powrie 1891, 92, 275) gives very important confirmation of this view. It is a young individual very perfectly preserved, the palato-quadrates and Meckel's cartilages are unossified, but the splint along the lower border of the latter and the entire series of opercular rays are present in their natural positions undisplaced. The dermal ossifications in the upper end of the hyoid arch are present in a normal manner, but below them, in a place usually concealed by the more laterally placed jaw elements, lie two superficial bones in the arch and a series of very short widely spaced opercular raylets. No other interpretation of these structures seems possible, and their presence affords conclusive evidence that the main operculum does not include the hyoid arch. Three branchial arches are represented each by a series of three or four slender rods concave upward so that their anterior extremities lay in the gill septum and their lower ends extended into the opercular fold.

The shoulder girdle contains a single element on each side. This is a bar of bone standing vertically on the side of the fish, with a narrow cylindrical upper end covered by the normal body scaling. Ventrally the bone expands into a powerful triangular structure whose ventral edge is rigidly articulated to the base of the pectoral fin spine on its inner and upper aspect. The lower part of the bone has an ornamented surface as if it had a series of large overlapping scales fused on to it; this surface was exposed freely on the flank of the fish. No supporting structures are to be seen associated with the spines of the other paired fins.

The main lateral-line passes along the flank, below the middle of its height, from the shoulder girdle to the tail. The ventral lateral-line is very well shown in C. 18 Zoo. Dept. Univ. Coll. It runs along the ventral surface from a point just mesial of the head of the pectoral spine to pass ventrally to the insertion of the intermediate spine.

ISCHNACANTHUS GRACILIS

Ischnacanthus gracilis is a common fish in the Lower Old Red Sandstone of Turin Hill. It is supposed to be immediately recognizable because it is the only Acanthodian fish in these deposits in which the jaws are beset with conspicuous teeth. The character of these teeth has been repeatedly described, among others by SMITH WOODWARD and BASHFORD DEAN. SMITH WOODWARD (1915) records that the dentition of *Ischnacanthus* is remarkable in that it includes at least one whorl of fused teeth at the anterior end of the mouth.

So far as I can make out the structure of *Ischnacanthus* in the true sense is as follows. The fish is fusiform and probably a little laterally compressed. Its deepest point lies at some distance behind the head in the region of the first dorsal fin where it is, perhaps, in a large specimen about a sixth of the total length. Smaller individuals appear to be more slender. The head ends in a bluntly rounded snout which projects little if at all beyond the extremity of the lower jaw. The orbit is of medium size and lies very anteriorly so that, as in *Mesacanthus*, the olfactory organ must have been exceedingly small.

The two dorsal fins lie, one rather anteriorly and somewhat in front of the pelvic fins, the other posteriorly and a little behind the anal. Each is supported by an anterior spine, that of the anterior fin usually being somewhat shorter than the spine of the posterior fin, and more distinctly curved. It is deeply grooved and ridged, the deepest groove lies anteriorly, the anterior margin forms a rounded ridge followed by a series of two or three more, less deeply impressed. The ridges separating these grooves have a smoothly rounded cylindrical surface. The spines are deeply inserted into the body in contrast to the entirely superficial attachment in more primitive Acanthodians.

The caudal pedicel is comparatively deep, at its smallest point about half the maximum depth of the fish. The dorsal border of the caudal region is only slightly raised dorsally and extends to a very narrow elongated point. The lower lobe of the caudal fin forms a large triangle terminating, as does that of *Mesacanthus*, in a peculiar recurved hook covered by a double row of large scales.

The anal fin is almost a duplicate of the second dorsal, possessing a similar nearly straight anterior spine. The pectoral fin spines are the largest in the fish and they are quite markedly curved. Structurally they greatly resemble those of the median fins, and are remarkable because of their hollowness. They contain a cavity which is relatively very large and extends along them for at any rate three-quarters of their

total length. There are no intermediate fin spines. The pelvic fin spines are short, nearly straight, broad in proportion to their length, and lie close together a little behind the point of insertion of the first dorsal fin.

Only traces of the fin membrane are to be seen, and they are found commonly only in the case of the anal and second dorsal fins, though I have seen fragments associated with the pectoral and pelvic fins. In the great majority of specimens there are no traces whatsoever of normal fin rays, the whole membrane being covered with exceedingly small square scales, similar to those covering the whole body, and arranged in lines parallel to the anterior spine. But Powrie 1891, 92, 254, a rather small fish, is unique in that both pectoral fins are supported by long straight unjointed fin rays, arranged apparently in two layers. These end some distance away from the

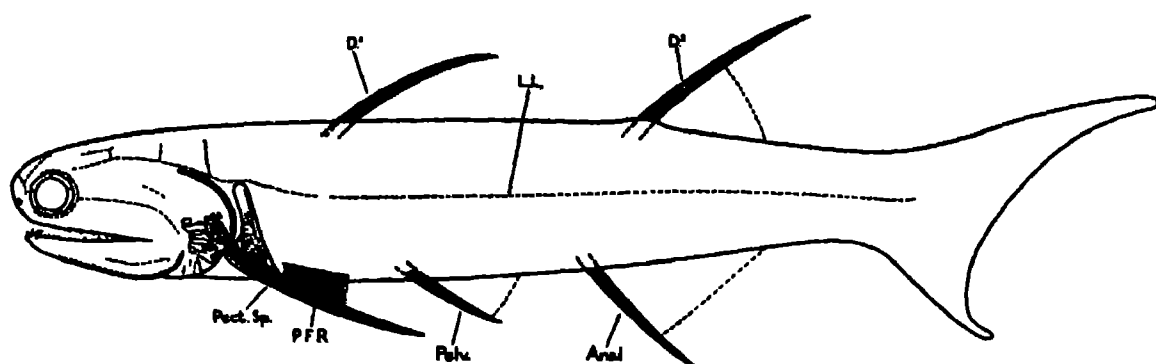


FIG. 10—*Ischnacanthus gracilis* EG. $\times 1.5$ approx. Reconstruction of the fish founded on medium-sized individuals from the L.O.R.S. of Turin Hill. Anal, anal fin; D^1 , D^2 , the dorsal fins; L.L. lateral-line; P.F.R. pectoral fin rays; Pect.Sp. pectoral fin spine; Pelv. pelvic fin.

scapula and are about as long as that bone. They are "bony" very slender rods, apparently of oval cross-section, and exactly resemble the corresponding structures of *Acanthodes*.

The body of *Ischnacanthus*, like that of every other Acanthodian, is covered with a continuous pavement of exceedingly small, square ganoid scales. These are proportionately smaller in *Ischnacanthus* than in *Mesacanthus*, being just under a quarter of a millimetre square in a fish whose total length is at least 4 cm. There is no evidence of the occurrence of enlarged scales at the point of insertion of any unpaired or pelvic fin. But the dorsal and ventral borders of the caudal fin are strengthened by a series apparently of one median and two lateral rows of enlarged scales, which are about twice as wide as those covering the body, and which seem to end abruptly a little in front of its termination. The anterior portion of the hypocaudal lobe of the caudal fin has a small area of square scales of similar size, the only considerable occurrence of such scales in the entire fish. The rest of the hypocaudal lobe is covered with scales varying in size from one-sixth of a millimetre down to about one-eleventh. The fin membranes are covered, wherever they can be seen, with square scales which usually,

even at the base, are distinctly smaller than those covering the body, and which exhibit a steady diminution in size toward the margin of the fin, ending in mere granulations whose characters can scarcely be seen. It is impossible to be certain whether the fin web extended to the extreme tip of the spine. In the case of the pelvic fin it seems to have done so, in the second dorsal and the anal fin it does not. All the fins are long-based, the margin of the web subsiding quite gradually into the trunk.

No trace of the neural cranium is to be seen in any specimen, but the powerful ossifications associated with the palato-quadrate and Meckel's cartilage are always conspicuous. In one specimen these bones had become disarticulated and are now preserved as exquisitely sharp moulds, squeezes from which display their structure to perfection. The lower jaw, as shown in this material and seen from its inner surface, has the normal outline of a shark's Meckel's cartilage. The articular surface for the quadrate is convex and placed obliquely so that its outer end lies posterior to its inner. The jaw is deep behind, with a rounded lower margin which is not turned outward, and tapers gradually anteriorly, terminating in an irregular and badly preserved expansion which shows some evidence of a pit which served, presumably, for the attachment of a ligament. It is quite clear that the anterior part of the lower jaw was ossified as a separate element from the posterior, but that in the relatively large specimen on which the present account is based, the two portions had become connected by a continuity of ossification along the lower border. The inner surface of the jaw, which alone is shown, has toward its upper margin a smooth and polished surface carried on a thickened rib, readily separable for the greater part of its extent. This surface bears a quite irregularly distributed series of denticles arising from it without any indication of a separately fused base. The upper surface of the lower jaw forms a flat shelf from whose labial edge arises an extremely deep ridge, which must be on the outer surface of whole bone. This ridge bears a series of small denticles some of which seem to be inserted in special depressed areas, recalling those within which the teeth of Osteolepid fishes and Labyrinthodont Amphibia are placed. Lying to the lingual side of the ridge, but completely confluent with it in the lower part at any rate, there is a series of relatively large, massive teeth with smooth pointed crowns. These stand on the flattened upper surface of the jaw, are perfectly continuous with the mass of the bone, and cannot be recognized as independent denticles. They are clearly unprovided with any mechanism for replacement. The dentition so described is restricted to the posterior element of the lower jaw and does not extend beyond it. This condition is recognizable not only in my perfectly preserved isolated jaws but also in complete heads. At the point where the two main elements of the lower jaw come into contact with one another there is a small triangular bone raised above the general level of the upper surface of the jaw, which may be a separate third element, but appears to be continuous with the ridge on the labial side of the jaw, to which the teeth are attached. I have not seen a satisfactory display of the outer surface of the jaw so that I cannot

say with certainty whether or no its lower margin was turned outward in the manner commonly found in Elasmobranch Meckel's cartilage, but I do not think that it was.

The palato-quadrate bones of the disarticulated skeleton are perfectly preserved and fortunately display both inner and outer surfaces. This bone is much shorter than the lower jaw, an indication that the palato-quadrate, like Meckel's cartilage, was ossified from more than one centre. This conclusion is drawn from the fact that the part of the palato-quadrate preserved corresponds quite accurately in length to the posterior portion of the lower jaw. The palato-quadrate bone so far as it is shown consists of a very deep post-orbital ramus formed of a thin film of bone which stood vertically in the whole skull, and an exceedingly short abruptly truncated palatal portion lying quite horizontally and very thin dorso-ventrally. The posterior part of the bone toward its anterior end bears at its lower margin a ridge projecting labially which is the palatal ramus, this subsides on to the outer surface of the ventral plate so as to leave space for masticatory muscles. The hinder margin of the bone turns out, the lower border of the everted portion being thickened to form the quadrate articular surface. From the quadrate forward the upper part of the bone forms part of a circle until it ends at a nearly vertical but somewhat irregular anterior border, which passes down to the palatal part of the bone. The tooth-bearing border of the palato-quadrate forms a narrow, wedge-shaped area, widest anteriorly where it forms a strong rod-like lower border to the bone, decreasing in width as it goes back. On the outer surface this area is bounded by a high band of bone, exactly similar to that occurring in the lower jaw. This bears a series of small teeth on its summit, the series being interrupted by the large teeth which are rooted on the surface of the jaw and rise from it with their outer surfaces in complete continuity with the labial flange. The ventral portion of the admedian surface of the palato-quadrate bone, like the corresponding strip of the lower jaw, is beset with tiny denticles, completely fused with it, which exhibit an irregular arrangement in rows parallel to the borders of the mouth, the teeth being ranged in threes, a larger between two considerably smaller denticles.

Many specimens of *Ischnacanthus* show a single whorl of large teeth placed quite anteriorly in the symphysis of the lower jaw. The largest tooth usually points almost directly backward into the mouth, and one or two lie in front of it. In B.M.N.H. 46305 a number of unattached sharp-pointed tooth crowns lie immediately behind the median whorl, the firmly attached teeth of the lower jaw only beginning much farther back. No similar teeth are to be found in the upper jaw. In the very large specimen Edinburgh 1887, 35, 2 a similar whorl consisting of four teeth of which the posterior is much the biggest is very well preserved, but there is not very satisfactory evidence of another similar whorl. In P. 311 of my own collection, a large fish which differs from *Ischnacanthus gracilis* in that the well-ossified jaw elements support only a very few small teeth, there are four independent tooth whorls, all displaced so that their original positions cannot be determined. The anterior part of the mouth in

1887, 35, 2 contains a vast number of exceedingly small toothlets about a quarter of a millimetre high. Each of these is like a *Diplodus* tooth with the middle cusp as long as the lateral ones, the three needle-like spines fanning out from a small base. The individual teeth are tightly packed together. These elements may be compared with those from Campbellton, New Brunswick, called *Doliodus* by TRAQUAIR (1893). These teeth are also shown in the normal-sized specimen B.M.N.H. 46305.

No trace of the hyoid arch is visible, but there is evidence in many specimens of ossifications associated with the branchial arches. These are best shown in P. 481 and P. 298 of my own collection. They are exceedingly slender, elongated, bony splints, attached to one another by their edges and extending from the upper part of the gill chamber downward and backward, at least to the level of the jaw articulation. Three branchial arches are recognizable. There is no doubt that these elements are homologous with those which, in the more primitive Acanthodians, *Climatius*, etc., lie in the skin of the outer surface of the gill septa and support the small opercula. In *Ischnacanthus* they are completely concealed by the mandibular operculum.

The shoulder girdle is represented by a single element on each side which seems to be, in part at least, of the nature of a membrane bone. It lies on the flank of the fish and bears no ornament. It is covered, at any rate for the greater part of its external surface, by the ordinary body squamation. The outer surface widens abruptly at its lower end, where it comes into contact with the upper and admesial surfaces of the pectoral fin spine, whose root, obliquely truncated and rounded, extends forward for a small distance in front of the shoulder girdle. The outer surface of the shoulder girdle turns inward at a line which is sometimes marked by a low outstanding ridge in such a way that it forms a narrow posterior surface to a gill chamber. The inner surface of the shoulder girdle is perfectly smooth and forms a cylindroid hollow, which must have lain in contact with the outer surface of a cartilaginous scapula. In one case, P. 298, there is present a mass of cartilage which lies in contact with the pectoral fin spine and with the inner surface of the shoulder girdle extending posteriorly to that element as a comparatively deep structure, which can only represent a mass of fused cartilaginous basals. The unusual specimen of *Ischnacanthus*, Edinburgh, Powrie 1891, 92, 258, shows a series of ossified basals in the right pectoral fin. These radiate from the point of attachment of the pectoral spine to the shoulder girdle and seem to be four in number; but none the less they present a remarkable resemblance to the basals of an Elasmobranch tribasal fin. The basals terminate at the proximal ends of the ossified fin rays. In another specimen, C. 4, there is some evidence of a calcified, cartilaginous pelvic girdle, associated with the base of the pelvic fin spine, but no details of structure can be given.

The outer surface of the head of *Ischnacanthus* differs somewhat in small (10 cm.) and large (25 cm.) individuals because the development of the ossicles on the cheek takes place late in life. The dorsal surface of the head has the normal Acanthodian structure, the typical body squamation of very small, square scales passing through a very short

transition region into the somewhat larger head scaling at a point about midway between the hinder border of the orbit and the articulation of the jaws.

The upper border of the gill chamber is formed by a double row of rather large oval overlapping scales, between which the main lateral-line runs forward from the body to pass above the orbit. Three incomplete commissures arise dorsally from this canal, one (B.M.N.H. 46305 and D.M.S.W. P. 481) just anterior to the posterior end of the gill

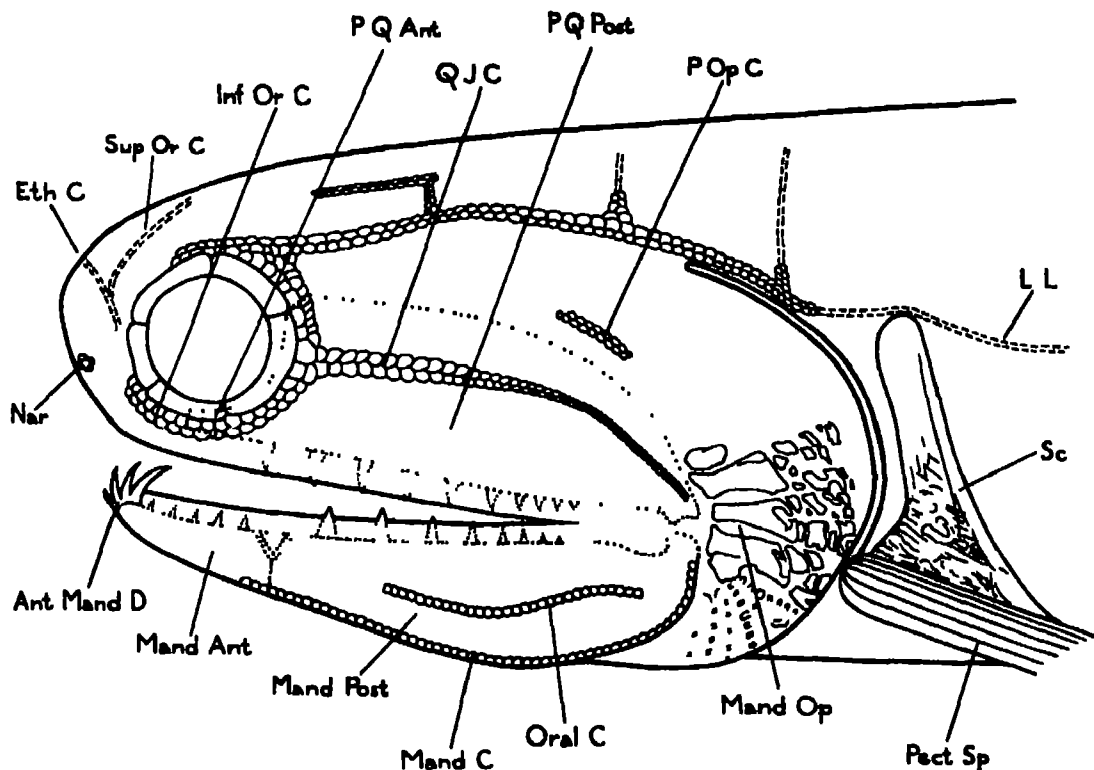


FIG. 11.—*Ischnacanthus gracilis* EG. $\times 4.9$. Reconstruction of head of a medium-sized individual from Turin Hill. *Ant.Mand.D.* the median whorl of teeth in the mandible; *Eth.C.* ethmoidal commissure; *Inf.Or.C.* infra-orbital canal; *L.L.* lateral-line; *Mand.Ant.* anterior bone in Meckel's cartilage; *Mand.C.* mandibular canal; *Mand.Op.* mandibular operculum; *Mand.Post.* the posterior mandibular bone; *Nar.* nostril; *Oral.C.* oral canal; *P.Q. Ant.* and *P.Q. Post.* the two ossifications in the palato-quadrate cartilage; *P.Op.C.* pre-opercular canal; *Pect.Sp.* pectoral spine; *Q.J.C.* quadrato-jugal canal; *Sc.* scapula; *Sup Or.C.* supra-orbital canal.

chamber, the second (Edinburgh 1887, 35, 2), just in front of the articulation of the jaws, is bordered by typical oval scales, whilst the third lies about half-way between the posterior border of the orbit and the articulation of the jaws and is shown as a deep groove between the small thick bones of the skull roof in the transition region from the body squamation. There is some indication of a pair of lateral-line grooves passing forward from the inner ends of these commissural grooves. The bones of the top of the head are small irregular elements arranged in rough antero-posterior rows as far forward as a deep transverse lateral-line groove which crosses the head at the anterior

border of the orbit, connecting the anterior ends of the main canals. From the points of junction a pair of badly marked grooves runs backward and inward, approaching one another above the posterior border of the orbit. The rounded anterior end of the head is covered by small thick scales, amongst which are some with rounded margins surrounding the nostrils, which lay probably close together and directed forward at the level of the centre of the orbit. The nostril is perfectly shown in a specimen belonging to the Dundee Natural History Society. It is a small circular hole placed far above the border of the mouth and forms the centre of a series of concentric rings of dermal bones.

The orbit is surrounded by the customary ring of very delicate though ornamented circum-orbitals, whose number appears to be five. This ring is itself framed by a double series of oval lateral-line scales arising from the main canal and extending continuously round the posterior and lower borders of the orbit. Its anterior end is not certainly determinable though it seems to cross the snout below the nostril.

The cheek is comparatively little ossified in small individuals but becomes completely scaled in large ones. It contains a typical double row of oval lateral-line scales arising from the post-orbital canal at about the level of the middle of the orbit, and extending backward to the jaw articulation. The rest of the cheek is when fully ossified completely covered by irregular elongated ornamented scales forming rough ridges and grooves. These in turn pass upward and backward, with no break whatsoever, into the mandibular operculum. The scaling of the cheek in front of the jaw articulation is feeble, but that of the lower jaw, composed of thick ornamented scales, is sometimes well shown. The ventral margin of the lower jaw is formed of a double row of scales bordering its main lateral-line, which is continuous over the articulation with the "pre-opercular" canal, and extends forward apparently to the symphysis. The more dorsally placed "oral" canal passes forward over a series of oval lateral-line scales toward, but not as far as, the anterior end. It appears to arise from the main canal.

The mandibular operculum, unlike that of the fish previously discussed, covers the whole gill chamber. The dorsal part is supported only by a series of very small elongated scales, of extreme tenuity in the small individuals, but becoming quite massive in the large specimens. These are arranged in irregular rows passing downward and backward. A group of short more massive bones, representing the opercular rays of other forms, lies just behind the jaw articulation. Immediately ventral to these, that part of the operculum which lies against the base of the scapula contains a relatively massive group of polygonal scales. The intergular part of the operculum has small isolated scales posteriorly, and seems to be naked in front.

The main lateral-line canal can be traced along the flank as a ridge rather above the middle line on the outer surface of the squamation running into the tail, where it continues along the dorsal lobe close to the lower margin, becoming less and less definite as it approaches the tip. The squamation seems to show extremely little change of character over the lateral-line canal, which is in fact only recognizable along

the flank by the presence of this well-defined ridge. This condition can only imply that the actual canal ran in a series of special ossifications lying below the scales and perhaps fixed to their lower surfaces. It is quite clear that the normal Acanthodian condition of the canal running between rows of scales does not apply to *Ischnacanthus*, even though it does to *Acanthodes*. There is a ventral line on the trunk which can usually be seen running backward just mesial of the base of the pectoral fin spine.

CHEIRACANTHUS

Individual fish belonging to the genus *Cheiracanthus* are very common in all the well-known Middle Old Red Sandstone nodule localities in the Moray Firth, at Achanarras, and in Orkney. Two species, *C. murchisoni* Ag. and *C. latus* Eg., are to be distinguished in the Moray Firth materials; they are identical in their morphology but differ in proportion and in some details. The following account distinguishes between them where necessary.

The fish are usually complete, but the scales on the body, unlike those on the fins, are usually displaced, and the exoskeleton of the head is always confused. The fins are very unusual amongst Acanthodians because the outline of the web is usually well preserved. It seems clear that the fish were round bodied and very heavily built. Evidently the head was wide from side to side and the pre-orbital part exceedingly short. The branchial region is short and deep. The body is deep in *C. murchisoni* and even deeper in *C. latus*. There is a single dorsal fin. The fin spines are always deeply inserted into the body. The caudal fin is very large, the upper lobe being upturned whilst the triangular lower lobe has a considerably deflected anterior margin. The caudal pedicel is deep. In *C. latus* the tail is enormous. The anal fin is like the dorsal but lies a little behind it. The pectorals are large and the pelvics also of great size, as large as the dorsal and anal.

The body is completely covered with a normal very fine squamation of very small square scales ornamented by longitudinally running ridges. There are no enlarged lateral-line scales, and none elsewhere on the body. The scales on the webs of the fins are arranged in rows and may be less than one-tenth of a millimetre square.

Many specimens of *Cheiracanthus* preserve an ossified chondrocranium. The ossification appears to have been continuous throughout the neural cranium, merely a thin film over the outer surface of the persisting cartilage as in the homologous structure in *Acanthodes*. The brain case is thus always crushed flat, and little beyond its main outline can be distinguished. It is evident that in general morphology it agreed closely with that of *A. bronni* described later in this paper. The region of the olfactory chambers is entirely unossified, as is the supra-orbital region above and in front of the orbit. The trabecular region forms a narrow bar, showing that the skull was tropibasic. This bar suddenly widens laterally at the anterior end of the otic region, where it is probable that it had facets directed forward for articulation with the anterior part of

the palato-quadrate. The otic region is very broad, and like that of an Actinopterygian has nearly parallel lateral margins forming strong ridges over the horizontal semi-circular canals. These extend backward from a post-orbital process for a long distance. It is probable that the otic process of the palato-quadrate articulated with the hinder surface of the post-orbital process. The character of the occipital region is not shown.

The palato-quadrate cartilage is completely ossified and shows no sign of origin from independent centres. The very long paraotic part of the bone is high, its upper border, which is segmental, being turned outward for the attachment of the masticatory muscles. The hinder end of this ridge is thickened and transversely widened at its

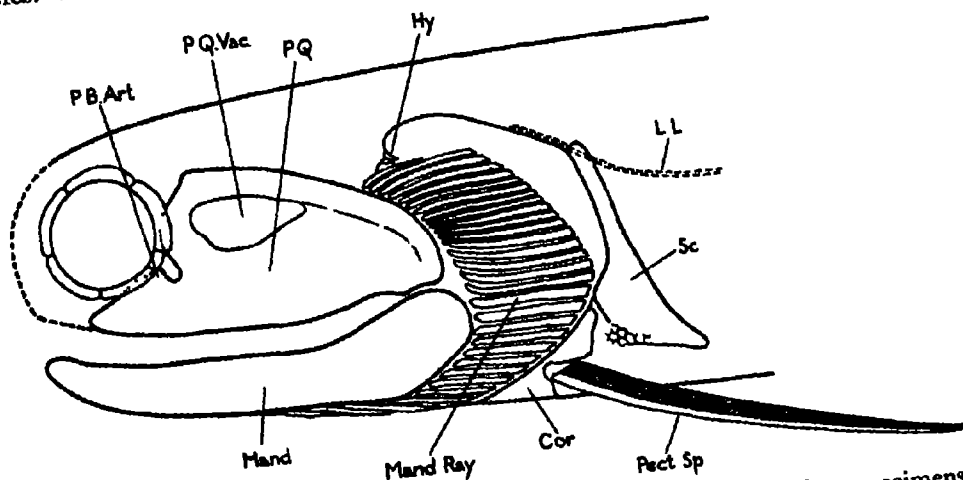


FIG. 12—*Cheiracanthus munchisoni* AG. $\times 3.4$. Reconstruction of head, mainly from specimens from the M.O.R.S. of Gamrie. Cor. coracoid; Hy. upper end of the hyoid arch; L.L. lateral-line; Mand. lower jaw; Mand.Ray, a ray of the mandibular operculum; P.B.Art. palato-basal articulation of the palato-quadrate; P.Q. palato-quadrate; P.Q.Vac. vacuity in the palato-quadrate; Pect.Sp. pectoral fin spine; Sc. scapula.

lower end to form a condyle for articulation of the lower jaw. The paraotic ramus ends anteriorly in a generally vertical everted border which finishes abruptly by forming the hinder margin of a deep notch on the admesial edge of the palatal ramus. The upper part of the anterior border of the paraotic plate slopes a little backward and ends in a small rounded anterior otic process which seems to have articulated with the post-orbital process of the neural cranium. The paraotic plate is perforated by a large, usually pear-shaped vacuity (originally found by JAEKEL) lying just within the thickened and everted border.

The short and wide palatal ramus lies nearly at right angles to the paraotic plate and has a blunt anterior point. Its lateral margin is continued backward on the outturned lower margin of the paraotic plate so as to leave only a very shallow bay for the masticatory muscle. The inner border of the palatal ramus ends abruptly in a backwardly facing knob, which lies mesial of the root of the anterior edge of the paraotic

plate, and by comparison with *Acanthodes* appears to have articulated with the basis cranii. It is evident that the palato-ptyergoids of the two sides were rather widely separated in front. The palato-quadrate of *Cheiracanthus latus* is much deeper than that of *C. munchisoni* but otherwise agrees with it.

Meckel's cartilage is elasmobranch-like in its general character. It is ossified as a single continuous bone. It is very slender in small specimens, becoming deeper in larger individuals, and has a very gently concave oral border. The anterior end is shallow and meets its fellow in symphysis. The articular surface lies on the upper side quite posteriorly, and has a small "coronoid" elevation immediately in front of it. The lower border of the posterior part of the structure is turned outward. It is evident from many specimens (P. 486, D.M.S.W. etc.) that the lower jaw is considerably longer than the palato-quadrate.

The cerato-hyal (figured by JAEKEL 1925) is usually visible as a straight cylindrical rod with an inturned anterior end which approaches nearly to that of its fellow, there being in my material no trace of hypo- or basi-hyals. The small specimen, Edinburgh 1884, 60, 3, suggests the presence of at least two pairs of ossified cerato-branchials. I have seen no evidence of ossified epi-hyals in any specimen.

The opercular fold is supported by a wonderful series of rays. On the ventral surface between the lower jaws there are two groups, each containing about eight very slender elements of great length, the longest of them being probably nearly half the entire length of the lower jaw. These all end anteriorly in a transverse plane or nearly so, and their posterior ends form part of a segment of a circle extending back to a point rather behind that of the articulation of the lower jaw. They lie parallel to one another and to the lower border of the jaw, and pass apparently without interruption into the more flattened rays attached to the lower jaw posteriorly. The rays, seven or eight in number, continue uninterruptedly into a series of some seventeen, which lie like a fringe attached to the hinder border of the palato-quadrate. These opercular rays are massive structures, each with a flat internal surface, and at any rate in the central part of the series each bears a strong longitudinal ridge which bifurcates before it reaches the distal end of the bone. The individual rays have nearly parallel margins by which they are in contact. The attached anterior ends of those rays which belong to the mandible are deflected, a single ray at the articulation of the jaws is straight and widened, whilst the pointed ends of the ventral rays of the palato-quadrate point upward. In *C. munchisoni* the rays are massive and in contact with one another to the dorsal end of the series, in *C. latus* the upper rays are slender and their anterior ends turn sharply downward. The series extends very far up the dorsal border of the palato-quadrate and at first it seemed probable that the whole of the regularly curved hinder end of the series rested on the border of the gill chamber. When, however, the head is reconstructed it becomes evident that only the lower part of the series up to the level of the articulation of the lower jaw can have done so, and that there remains a narrow but deep area which cannot have been covered by the ossified portion of the mandibular operculum.

In several specimens (D.M.S.W. P. 492, B.M.N.H. 43273, P. 3203, 36010, and others) belonging to both species, the mandibular operculum has been dragged a little downward and there is shown a series of dermal ossifications belonging to the branchial arches. These individual bones are long slender rods which overlap one another and clearly lay in the skin on the outer side of the gill septum. They are always a little displaced but three independent arches are recognizable. It is most probable that they are the first three branchial arches, the hyoid arch being without ossification. In B.M.N.H. 43273, where the upper border of the gill chamber is unusually well shown, two small sickle-shaped bones projecting down from it correspond very well with the bones which lie in the upper end of the hyoid arch in *Climatius*.

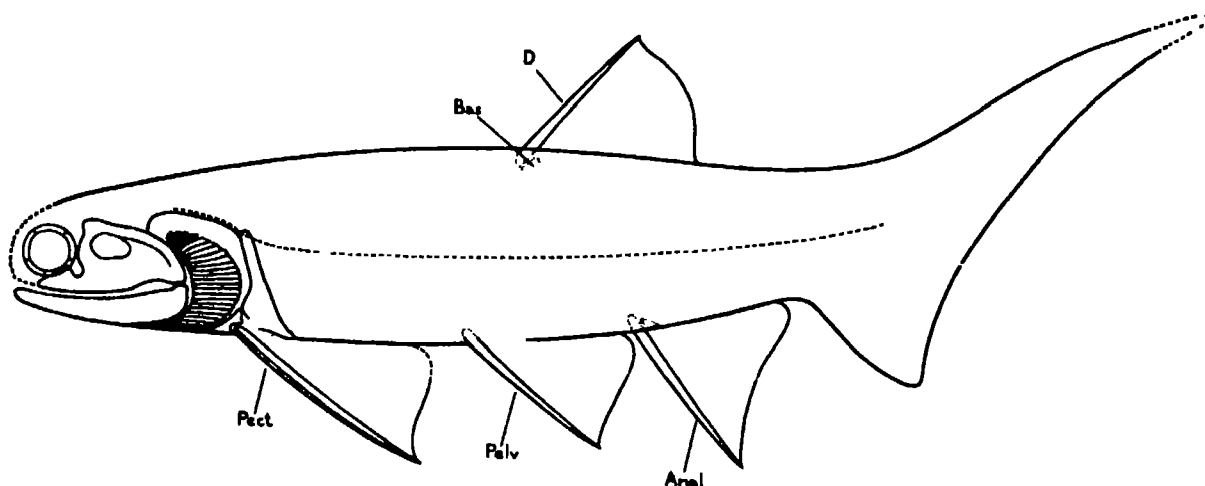


FIG. 13—*Cheiracanthus latus* EG. $\times 1.0$. Reconstruction of fish from specimens from the M O.R.S. of Tynet Burn and Gamrie. *Anal*, anal fin; *Bas*, basal of dorsal fin *D*; *Pect*, pectoral fin; *Pelv*, pelvic fin.

The exoskeleton of the head is very badly shown. The circum-orbital ring of plates though ornamented is feeble, the individual bones being narrow and thin. They seem in D.M.S.W. P. 485 to be six in number, but can seldom be counted. The upper surface and snout are completely covered by a mosaic of small polygonal scales whose arrangement cannot be determined. There is some evidence of a small nostril lying rather high up on the front of the head. It is probable that the cheek and lower jaw lacked ossification, though there is a series of small scales below the eye.

The main lateral-line is sometimes shown on the flank, but no other parts of the apparatus can be seen.

The shoulder girdle consists of two bones on each side, neither of which bears any ornament but lies underneath the scale-containing skin. The upper bone, the scapula, has a narrow upstanding process containing an hour-glass-shaped cavity formerly filled with cartilage. Ventrally this bone widens, its cavity becoming very large so that the bone is always crushed. It seems, however, to have been flattened in life. The

ventral bone is also hollow, a shell of dense bone surrounding a clear calcite infilling which was no doubt cartilage during life. It lies anterior to and below the scapula and may be called coracoid. Its outer surface is deeply grooved for the reception of the base of the spine and its lower edge is turned outward.

The spine is slightly curved, and has a smooth anterior edge separated from the flat surfaces by grooves on each side so deep that the whole front of the spine looks like an independent cylindrical thread. The spine contains a cavity reaching nearly to its tip.

The dorsal fin of *Cheiracanthus latus* commonly, and its anal fin occasionally, possess small basal bones, single elements tightly attached to the extreme base of the fin spine on its posterior surface.

DIPLACANTHUS STRIATUS

Diplacanthus striatus is a fish found frequently in all the Middle Old Red Sandstone nodule localities surrounding the Moray Firth, but is especially common and well preserved at Tynet Burn and Gamrie. It also occurs in abundance at Achanarras in Caithness, and in the Stromness Beds in Orkney.

One single specimen, Powrie 1891, 92, 334, Royal Scottish Museum, which is certainly determinable from the character of its fin spines, shoulder girdle, scales and mandibular splints, differs from all other specimens of *D. striatus* in that it has very massive ossifications in the head skeleton, vertebral column and basals of the fins, and possesses well-preserved dermal fin rays. Other specimens show slight traces of ossification of the same nature in the branchial arches, thus confirming the identification. The causes of this very extensive ossification in this individual are quite unknown. The specimen is not of exceptionally large size.

The normal length is about 9 cm. The dozen specimens before me are preserved dorso-ventrally flattened or in profile in almost equal numbers. This arrangement may depend more on the fact that both the dorsal and ventral fin spines are very long than on the real shape of the body of the fish, but when taken in conjunction with other evidence derived from the shoulder girdle it goes to show that the height and width of the fish at the shoulder were about equal, and that the animal possessed a flattened ventral surface.

It is clear that the fish was rather abruptly truncated in front, the snout being rounded from side to side and probably somewhat wedge-shaped dorso-ventrally; the exact profile is, however, impossible to determine. When allowance is made for the widening due to crushing the fish is comparatively slender and stream-lined, though the caudal pedicel is deep.

The caudal fin is heterocercal, its upper lobe appearing to turn upward somewhat more than in the Lower Devonian Acanthodians and the lower lobe forming a less sharply marked triangle than in those fishes. The posterior border of the web of the caudal fin forms only a very shallow bay.

The dorsal fins of typical Acanthodian pattern are supported by enormously tall fin spines, of which the anterior is curved and the second, somewhat shorter than the first, is inserted at an angle of about 60° with the body surface and is nearly straight. The anal fin has a straight very long and slender spine about the length of the second dorsal, and lies posteriorly to it. The web of the dorsal fins is occasionally preserved, at

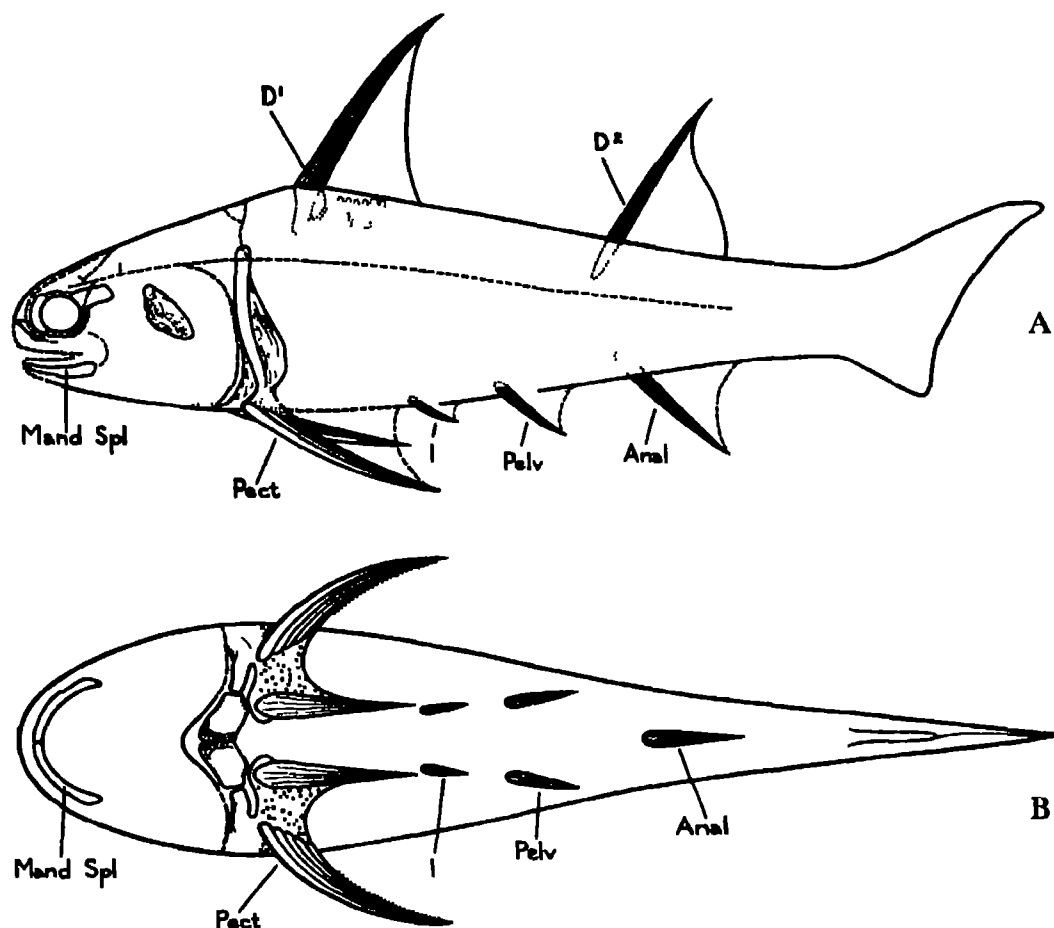


FIG. 14—*Diplacanthus striatus* Ag. $\times 1.5$. Reconstructions of (A) left lateral and (B) ventral surface, founded on specimens from Tynet Burn and Gamrie, M.O.R.S. *Anal*, anal fin; *D¹* and *D²*, dorsal fins; *I*, intermediate spine; *Mand.Spl.* mandibular splint; *Pect.* pectoral apparatus; *Pelv.* pelvic fin.

any rate in part, and seems to have a very short attachment along the body and not to extend to the extreme tip of the spine. The spines of the dorsal fins are remarkable in that, unlike those of *Mesacanthus* and *Ischnacanthus*, they have a long inserted portion. The exerted part bears an ornamentation of coarse longitudinal ribs parallel to its anterior margin, the insert part narrows rapidly and is longitudinally striated. Each spine is hollow, the cavity extending almost to its tip and lying rather posteriorly in the spine.

The unique specimen, Edinburgh, Powrie 1891, 92, 334, shows a series of three well-ossified basals in the first dorsal fin. Of these the first has its anterior margin clasped by the split hinder surface of the spine, the second is larger, and the third of considerable size. There is definite evidence of the existence of a uniform row of small cylindrical radials in the base of the fin, and some indication of additional bones between these and the three basals. This fin is supported throughout its visible extent by ceratotrachia, delicate cylindrical rods of uniform diameter which are obscurely grouped into bundles ending at the level of the dorsal ends of the radials. This specimen and others show that the surface of the fin web is coated with a mosaic of normal square scales of very small size.

There are three pairs of fins. The pectoral, which in normal specimens never shows any trace of a web, possesses two spines and an elaborate shoulder girdle which will be described later. The lateral spine is long, somewhat curved and widening at its base and shows a marked distinction between exerted and inserted regions, like the dorsal-fin spine. The admedian spine lying parallel to the principal plane of the animal is of such a length that its tip lies a little anterior to that of the lateral spine; it is powerful and deeply ridged and grooved. The very short and delicate spines of the intermediate fins lie immediately behind the posterior ends of the admedian spines of the pectoral fins. The pelvic-fin spines, which lie a short distance behind those of the intermediate fins, are twice as long as the pair in front of them. They are relatively wide and powerful. In C. 15, and in Powrie 1891, 92, 334, there is a trace of the web of the pelvic fins, but it is not sufficiently well preserved to show any details.

The whole body behind the head region is covered with normal square Acanthodian scales, about a quarter of a millimetre across. The fin webs sometimes possess similar squarish scales of much smaller dimensions. The lateral-line runs between two rows of somewhat enlarged scales, the straight obliquely set rows of scales being broken at its level. It lies nearer to dorsal than the ventral margin of the fish, but is often very difficult to follow in the material. The mid-dorsal region of the body immediately in front of the first dorsal fin is covered with a development of rather large irregular scales similar to those covering the top of the head, but separated from them by an area of normal small square body scales.

The endoskeleton of the head is usually completely unossified but traces of the branchial arches are sometimes visible as bone, and Powrie 1891, 92, 334 shows the whole fully ossified but so crushed as to be almost incapable of interpretation. The neural cranium shows no intelligible features, but a deep palato-quadrate ossified in two sections—a small palatal part and a very deep quadrate region—and a similarly double Meckel's cartilage are visible. The other elements of the visceral skeleton are a series of extraordinarily massive rods which may be a cerato-hyal and cerato-branchials. Without the counterpart this specimen cannot be further discussed.

The vertebral column is ossified in Powrie 1891, 92, 334, but its elements are so involved with scales that it is not possible to give a detailed description. The structure

was notochordal throughout, but there is a series of rather wide-spaced neural arches. The corresponding subnotochordal bones seem, at any rate in the space between the pelvic and anal fins, to have been continuous across the middle line. There is no evidence of ossified ribs.

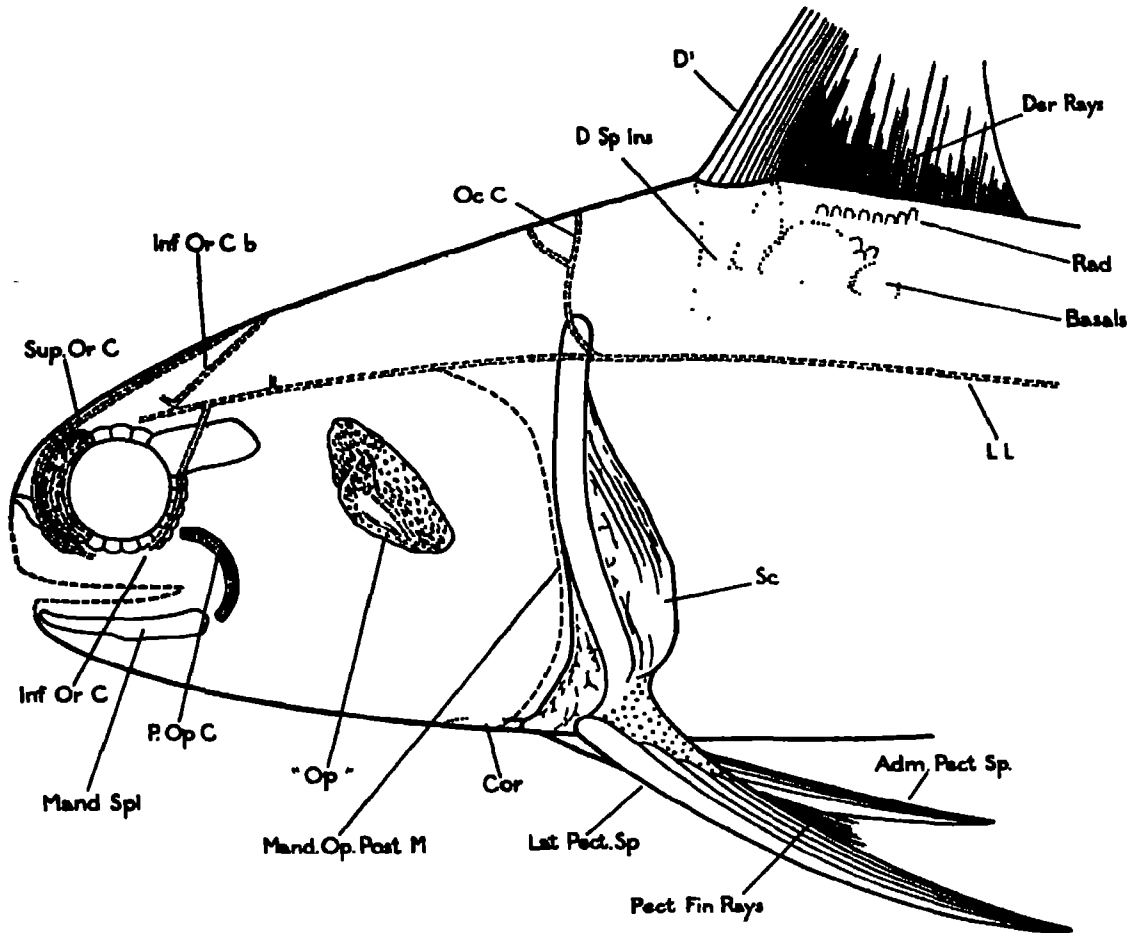


FIG. 15—*Diplacanthus striatus* AG. $\times 3.8$. Reconstruction of anterior part of body on the basis of specimens from the M.O.R.S. of Tynct Burn. *Adm.Pect.Sp.* admedian pectoral spine; *Basals*, of first dorsal fin; *Cor.* coracoid; *D¹*, spine of first dorsal fin; *D.Sp.Ins.* its inserted part dotted; *Der.Rays*, dermal rays, ceratotrichia, of first dorsal fin; *Inf.Or.C.* infra-orbital canal; *Inf.Or.C.b.* postero-median branch of the infra-orbital canal (STENSIO); *L.L.* lateral-line; *Lat.Pect.Sp.* lateral pectoral spine; *Mand.Op.Post.M.* posterior border of the mandibular operculum; *Mand.Spl.* mandibular splint; *Oc.C.* occipital commissure; "*Op.*" large bone in the operculum; *P.Op.C.* pre-opercular canal; *Pect.Fin.Rays*, ceratotrichia of the pectoral fin; *Rad.* radials of first dorsal fin; *Sc.* scapula; *Sup.Or.C.* supra-orbital canal.

The exoskeleton of the head is exceedingly well shown in D.M.S.W. P. 300. The normal regular squamation of the trunk is continued forward to the occiput where it passes into a transition region in which the individual scales become larger and are quite irregularly arranged. As they are traced forward these dermal bones become

larger and are arranged in irregular longitudinal rows of which there are about twelve in the orbital region. This coherent dorsal shield ends abruptly in front at a pair of large transversely placed bones, whose lateral and anterior corners are turned downward in front of the circum-orbitals. There is some evidence that another somewhat smaller bone continues the process downward.

The position of the nostril cannot be determined, but it certainly lies below and in front of this head shield in a region which has either no dermal bones or at most a sparse covering.

The whole structure of the circum-orbital chain can be made out from a comparison of many specimens. The orbit is circular, the postero-dorsal part of its border being carried on a large bone which is always recognizable because it is crossed by the upper end of the infra-orbital lateral-line canal. Below this bone the posterior and ventral border of the orbit is borne by a series of about nine small bones forming a continuous chain, which is in part enclosed by a similar external series, the lateral-line running between the two. The anterior border of the orbit lies in a large well-ornamented bone which is in contact, by its non-orbital margin, with the lateral border of the coherent head shield. The orbit is completed by a chain of three small bones lying lateral to the head shield.

The mouth is very short, and the lateral surface of the head below and behind the orbit is continuous with the outer surface of the large mandibular operculum from which it is indistinguishable. This surface in the middle of its height and length contains a single very large highly ornamented bony plate, but is otherwise covered with small rounded scales which in the region before the large plate and behind the corner of the mouth are vertically elongated and arranged roughly in vertical rows. In a single specimen (P. 299) a short chain of bones, of the kind always associated with a lateral-line canal, runs in a semicircle from the posterior side of the circum-orbital ring toward the hinder end of the lower jaw.

The free border of the mandibular operculum is sometimes shown, visible merely by the disappearance of the squamation, there being no series of bones defining it. These traces are, however, quite consistent and show that the operculum covered the whole gill chamber, its margins resting on the lowest row of the body scaling, on the shoulder girdle, and on the anterior border of a small triangular patch of large scales which extends forward from the shoulder girdle on to the ventral surface of the pharyngeal region. The operculum seems to be continuous across the mid-ventral line and its small squamation merges into that of the intergular space. The only individually recognizable bones in this region are the pair of mandibular splints which are shown in Powrie 1891, 92, 334 to be attached to the ventral border of Meckel's cartilage. They are extremely smooth dense bones of characteristic shape, their shallow anterior ends meeting in a symphysis; they deepen posteriorly and terminate in a blunt point.

The lateral-line of the head is in part shown to perfection in D.M.S.W. P. 300. As in many other Acanthodians the anterior part of the system is peculiar in that the

actual canal runs through a chain of short bony cylinders. These may be, and probably are, independent of the normal dermal bones. Usually the canal surrounded by the apparently single cylinder lies between two rows of dermal bones. Posteriorly these cylinders are absent and the course of the canal can only be determined from the two rows of scales, a matter of difficulty especially on the body.

The main canal lies on the flank, rather above its mid-line. It passes forward over the upper part of the scapula on which it leaves no impression and descends on to the margin of the gill chamber. There it continues, becoming surrounded by bony cylinders, immediately mesial of the large circum-orbital plate, until it ends above the

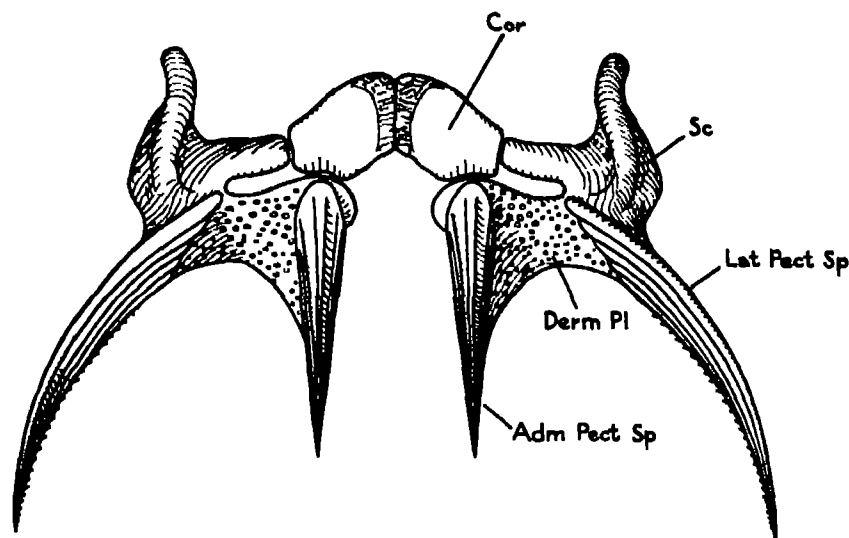


FIG. 16—*Diplacanthus striatus* Ag. $\times 3.6$. Reconstruction of the ventral aspect of the pectoral girdle. From Tynet specimens. *Adm.Pect.Sp.* admedian pectoral spine; *Cor.* coracoid; *Derm.Pl.* dermal plate connecting the pectoral spines; *Lat.Pect.Sp.* lateral pectoral spine; *Sc.* scapula.

middle of the orbit. Three dorsal branches arise from this canal, one lies in the region of the shoulder girdle crossing the middle line in front of the spine of the first dorsal fin and having an M-shaped course. The next lies above the hinder border of the large circum-orbital and passes directly inward for a short distance. The most anterior, the posterior branch of the infra-orbital canal, arises above the back of the orbit and passes inward and backward to meet, or nearly to meet, its fellow immediately behind the junction of the supra-orbital canals. The infra-orbital canal arises from the main canal some distance behind its anterior end. It then crosses the large posterior circum-orbital and continues downward below the orbit immediately external to the ring of small circum-orbitals. It cannot be seen to join the anterior end of the supra-orbital canal, but probably does so. A short canal appears to have arisen from the infra-orbital canal at about the level of the lower border of the orbit and proceeds over the cheek

to the lower jaw. The supra-orbital canal begins posteriorly in a junction with its fellow in the middle line; the two then separate, pursuing nearly straight courses, until each plunges downward immediately lateral to the large bone which forms the anterior end of the dorsal shield and mesial to the large anterior circum-orbital. It there continues, perhaps to join the anterior end of the infra-orbital canal. There is a slight indication of a ventral line on the body, running on the mesial side of the inner fin spines of the shoulder complex and the intermediate fin spines.

The shoulder girdle of *Diplacanthus* was first described by SMITH WOODWARD (1891), to whose account I can add little. It consists of an element standing vertically, parallel to the flank and about on the posterior border of the gill chamber. This bone, the scapula, is perforated from top to bottom by a cavity, which widens posteriorly at the place where the lateral pectoral spine is inserted. The analogy of all other similar structures in Acanthodians shows that this cavity, which is filled with clear transparent calcite in the fossils, represents a cartilage, and it is completely surrounded by bone. Posteriorly this bone forms a wide lappet which extends down to the insertion of the spine and is ornamented with a series of shallow grooves parallel to its posterior margin. It certainly lay in the skin of the flank. The ventral end of the bone widens in an antero-posterior direction and is attached to the lateral pectoral fin spine for a long distance, fitting on to it by a flat surface which rests on a definite close-fitting and grooved rabbet. The anterior border of the ventral part of the bone turns inwards at right angles to the length of the fish, and quite clearly forms part of the posterior wall of the gill chamber. Here it is smooth, in contra-distinction to the ornamented outer surface of the bone. The wide base of the lateral fin spine is held on its dorsal surface by the expanded lower end of the dorsal shoulder-girdle bone. Its ventral surface exhibits a sudden transition from the coarsely ribbed exsert to a finely striated insert region, which was in life covered by a V-shaped dermal bone (*Derm.Pl.*), connecting together the lateral and admedian spines. This bone bears a well-defined ornament of tubercles. The admedian spine, exceedingly wide and powerful, is firmly held for three-quarters of its length by this dermal bone, and its admedian border also is surrounded by dermal bone which appears to be continuous with that which is interposed between the two spines. Finally, the shoulder girdle is completed by a pair of elements which seem to articulate both with the dorsal shoulder girdle and with the ventral dermal element surrounding the base of the admedian spine, and are themselves connected by some form of sutural union. It seems probable that these bones lay on the ventral surface because in specimen P. 299 they are continued forward by a triangular patch of large scales. These anterior elements which meet in the middle line are hollow and filled with clear crystalline calcite; in other words they are presumably perichondral bone laid down round a coracoid cartilage. The whole arrangement is unique and extraordinarily difficult to determine. The essential facts, however, are that the lateral-fin spine is firmly clasped between two independent bones; the admedian spine is inserted into a slot in one of these; and there is a third ventral element which

articulates with the other two. No supporting elements are to be seen in association with any of the other paired fins. The specimen Powrie 1891, 92, 334 shows a series of ceratotrichia associated with the pectoral fin.

ACANTHODES

The ironstone nodules from the Lebach shales contain a considerable number of specimens of *Acanthodes*. These are often exceedingly well preserved and our knowledge of the endoskeleton of Acanthodians has been based almost entirely upon them.

AGASSIZ in 1835 founded the genus *Acanthodes* on such materials, two poor specimens being figured as *A. bronnii*. In 1848 BEYRICH described a fish from the Rothliegende of Klein Neundorf as *Holacanthodes gracilis* and this was well described and figured by ROEMER in 1857 as *Acanthodes gracilis*. In the same year TROSCHEI published the first characteristic figures of *A. bronnii* AG., and in 1868 KNER gave further excellent figures of Lebach specimens, referring them to *A. gracilis*.

In the British Museum Catalogue SMITH WOODWARD (1891) groups all the Lebach and Rothliegende *Acanthodes* together under *A. bronnii*, and his views have been accepted by all subsequent authors.

It was therefore with considerable surprise that I found the numerous Lebach specimens forming the basis of the following account to differ very widely from one another in the proportionate sizes of their parts, and that the variations in, for example, the ratio of the length of the mandibular splint to that of the pectoral fin spine, may be 100 %, and cannot be accounted for by growth (see Table I).

TABLE I—LIST OF SPECIMENS OF *ACANTHODES* FROM LEBACH USED
IN THIS ACCOUNT

No. of specimen	Length in mm of mandibular splint	Length in mm of pectoral spine
B.M.N.H. P. 6192	15.0	15.5
B.M.N.H. 40050	13.0 plus	19.5
Berlin (unnumbered)	20.5	32.0
D.M.S.W. P. 496	24.0	40.0
D.M.S.W. P. 498	19.0 plus (< 28)	50.0 plus
D.M.S.W. P. 494	28.5	49.0 plus
Berlin (unnumbered)	29.5	29.0
B.M.N.H. P. 4477 a	29.5 (?)	50.0
Edinburgh 1891, 42, 3	32.0	—
Edinburgh (unnumbered)	35.0	45.0
B.M.N.H. 22658 a and b	37.0	35.5
B.M.N.H. 40049	52.0	61.0 plus
D.M.S.W. P. 493	60.0	—
D.M.S.W. P. 323	65.0	73.0 plus
Pollichia II	71.0	—

MISS TOWNEND has therefore made a series of very careful restorations of the anterior ends of several individual fishes, four of which are reproduced in fig. 20. It is evident

that these individuals must belong to different species in that they differ not only in proportion, but very strikingly in the extent to which ossification of the lower jaw has gone at a given size.

Except for the series of forms from the Gas Coal of Nyran described by FRITSCH, and for *A. rouvillei* SAUVAGE, no other names have been given to European Upper Coal Measure or Lower Permian Acanthodians.

It is obvious that AGASSIZ's two type specimens of *A. bronni* will never be capable of determination, and for the purposes of this paper it is unnecessary to introduce a number of new specific names for these fish. Therefore I shall simply refer when necessary to individual specimens, without specific attributions.

The smaller fish in these nodules are usually preserved complete except for the tail. They are, however, twisted, the anterior end of the body often forming a loop from which the hinder end stretches straight backward. The long cylindrical head is crushed into a plane usually in such a way that the flattened dorsal surface and the intergular space retain their full widths, the sides of the head being superimposed, one dorsal of the other by a distance equal to the width of the dorsal surface. This arrangement allows restorations of the head to be made easily and accurately, but results in a compression of the rounded anterior end of the head of such a kind that the structure in front of the orbits can never be understood.

The larger specimens are always represented by isolated portions, head, body and tail being in separate nodules. In my experience these heads are usually seen in lateral aspect, but REIS has figured a considerable number which are dorso-ventrally compressed. The appearance of these heads suggests that the skin, cartilages and ligaments were all intact at the time of burial whilst all other tissues had been destroyed, so that in dorso-ventrally flattened specimens the pharyngo-branchials rest directly on the cerato-branchials, a disposition which adds greatly to the difficulties of interpretation. The structures are, however, quite perfectly preserved, chiefly as moulds from which the remaining fragments of bone can be removed by treatment with dilute hydrochloric acid.

REIS in his elaborate description of *Acanthodes* gave an account of the neural cranium based on such part of it as could be seen in complete heads. According to his description it was composed essentially of two bony elements, a large thin shield dorsally and a remarkable T-shaped bone perforated by a large foramen lying on the ventral surface in the orbital region; this he called the trabecular. I am fortunate in that a single specimen, P. 495, shows the entire isolated neural cranium so far as it was ossified. Owing to the fact that a great deal of the side wall remained as cartilage, the ventral elements of this brain case sank down until they came into contact with its dorsal bones, but they are not otherwise displaced. The individual elements all consist of thin sheets of bone surrounding cavities which still exist as such and were in life certainly occupied by cartilage. The account that I give of them pays no attention to the cartilaginous hollow, the whole bone being described as though it were solid, or as if the description applied to the cast.

The base of the skull is composed of a series of three bones, the anterior and posterior of which are much more massive than that which lies between them, and are to be seen in other specimens. Seen from its ventral surface the posterior bone has a wide anterior and very narrow posterior end, its lower surface being gently convex and its lateral surfaces in the posterior three-quarters of the bone curving upwards almost at right angles to the ventral surface, indeed in the middle of its length being inrolled above it. Quite anteriorly the lateral surface flares somewhat outward and is deep. In

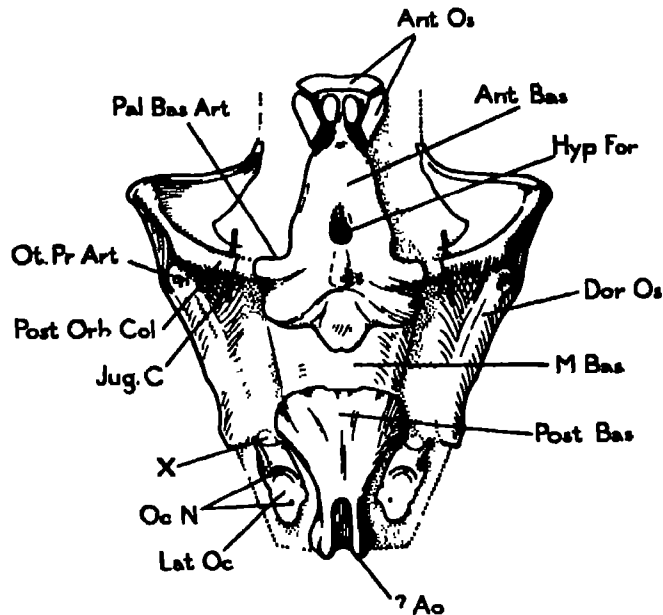


FIG. 17—*Acanthodes* sp. Lebach ironstones, P. 495, D.M.S.W. collection. Ventral aspect. $\times 1.2$. Parts shaded in line direct drawings, stippled areas restored. *Ant.Bas.* anterior basal; *Ant.Os.* anterior ossicles; ? *Ao.* groove for dorsal aorta?; *Dor.Os.* dorsal bone; *Hyp.For.* hypophyseal foramen; *Jug.C.* jugular canal; *Lat.Oc.* lateral occipital; *M.Bas.* middle basal; *Oc.N.* foramina for occipital nerves; *Ot.Pr.Art.* articulation for the otic process; *Pal.Bas.Art.* articulation for the palato-basal process; *Post.Bas.* posterior basal; *Post.Orb.Col.* post orbital column; *X*, notch for the vagus nerve.

the middle of the length it seems certain that the upper surface of the bone was carved out into a notch forming the lower part of a foramen and continued posteriorly by a shallow groove on the side of the bone. The posterior margin of the bone is deeply notched in the middle line, and a very well-defined groove, presumably for the anterior end of the dorsal aorta, runs forward from the notch until it suddenly ends, the bone in front of it descending abruptly to the ordinary level of the lower surface. The middle bone is a featureless structure shown to be exceedingly thin, but the anterior bone is very massive and has a most characteristic and recognizable shape. The large foramen which perforates it lies in the middle of its length and occupies one-quarter of the total width of the nearly flat lower surface at this point. Forward from here the bone

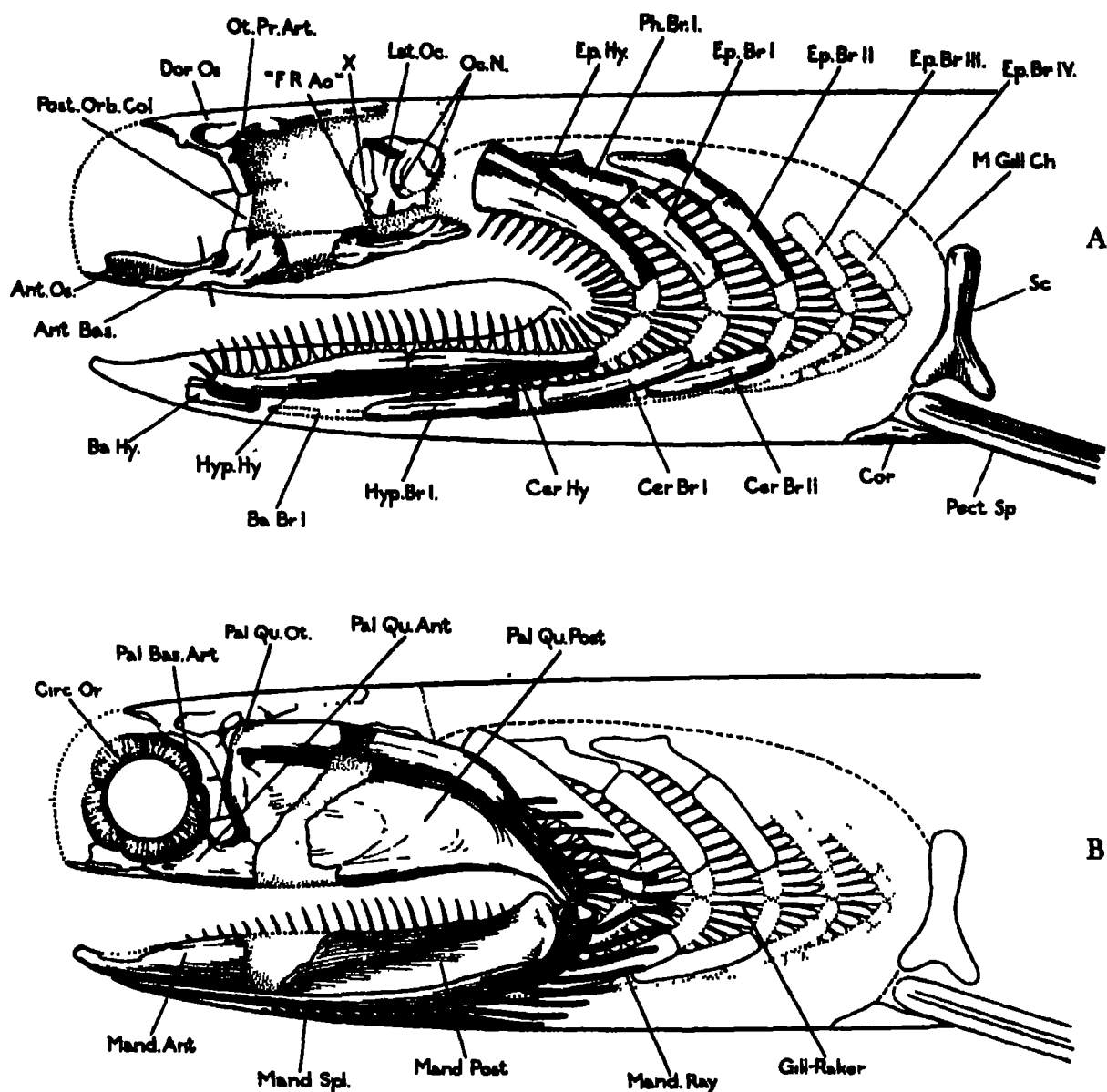


FIG. 18—*Acanthodes* sp. Reconstructions of the head, from specimens from the Lebach iron-stones, especially P. 495, P. 493, P. 323, D.M.S.W. collection; B.M.N.H. 40049, and Pollichia II. $\times 1.1$. A, with the mandible and palato-quadrates removed. B, complete except for the squamation. *Ant.Bas.* anterior basal; *Ant.Os.* anterior ossification in the basis cranii; *Ba.Br. I*, basi-branchial I; *Ba.Hy.* basi-hyal; *Cer.Br. I, II*, cerato-branchials I, II, etc.; *Cer.Hy.* cerato-hyal; *Circ.Or.* circum-orbital bones; *Cor.* coracoid; *Dor.Os.* dorsal bone in the neural cranium; *Ep.Br. I, II, III, IV*, epi-branchials I, II, etc.; *Ep.Hy.* epi-hyal; "*F.R.Ao.*" foramen for the radix aortae; *Gill-Raker*; *Hyp.Br. I*, hypo-branchial; *Hyp.Hy.* hypo-hyal; *Lat.Oc.* lateral ossification in the neural cranium; *M.Gill.Ch.* margin of the gill chamber; *Mand.Ant.* anterior ossification in Meckel's cartilage; *Mand.Post.* posterior ossification in Meckel's cartilage; *Mand.Ray*, ray of the mandibular operculum; *Mand.Spl.* mandibular splint; *Oc.N.* foramina for the occipital nerves; *Ot.Pr.Art.* articular facet for the otic process;

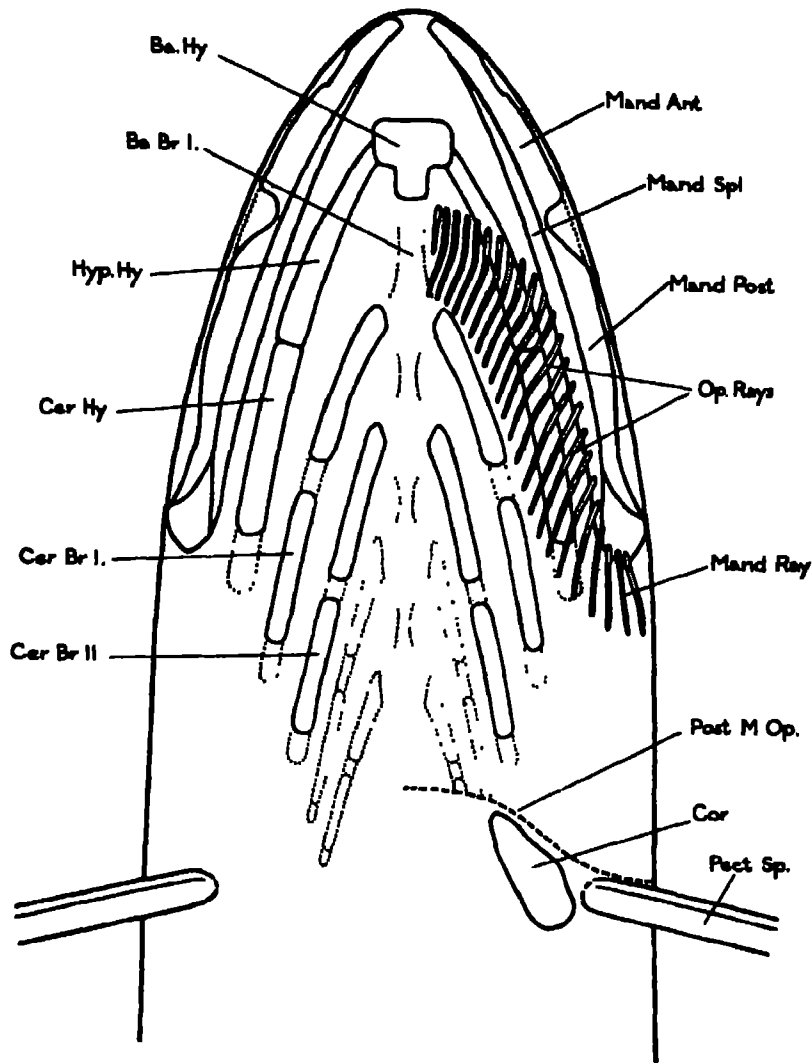


FIG. 19—*Acanthodes* sp. Lebach ironstones, reconstruction of the head, $\times 1.1$, from the same material as fig. 18. Ventral aspect with the squamation not shown, and the gill-rakers omitted. *Ba.Br. I.*, basi-branchial I; *Ba.Hy.* basi-hyal; *Cer.Br. I* and *II*, cerato-branchials I, II, etc.; *Cer.Hy.* cerato-hyal; *Cor.* coracoid; *Hyp.Hy.* hypo-hyal; *Mand.Ant.* anterior bone in Meckel's cartilage; *Mand.Post.* posterior bone in Meckel's cartilage; *Mand.Ray*, ray in the mandibular operculum; *Mand.Spl.* mandibular splint; *Op.Rays*, rays in the mandibular operculum; *Pect.Sp.* pectoral fin spine; *Post.M.Op.* posterior margin of the operculum.

Pal.Bas.Art. palato-basal articulation; *Pal.Qu.Ant.* anterior bone in the palato-quadrate cartilage; *Pal.Qu.Ot.* otic process of the palato-quadrate; *Pal.Qu.Post.* posterior bone in the palato-quadrate cartilage; *Pect.Sp.* spine of the pectoral fin; *Ph.Br. I.* pharyngo-branchial I; *Post.Orb.Col.* post-orbital column; *Sc.* scapula; *X*, foramen for the vagus nerve.

narrows gently until its lateral margins suddenly turn in to meet anteriorly in a blunt point. The foramen lies at the hinder end of a short shallow groove which travels forward in the middle line of the under-surface of the bone, and is for a persistent hypophysial duct. Immediately behind the foramen the lateral margins suddenly turn outward and pass upward as powerful bony transverse flanges. The flanges end abruptly, their lateral surfaces forming rounded ridges anteriorly which were obviously continued upward by cartilage in the complete neural cranium to meet a large process lying behind the post-orbital process on the dorso-lateral bone. Behind this ridge the surface of the flange is depressed and slopes dorsally at a somewhat higher angle than the ridge itself. On its upper surface this bone has a deep rounded ridge along the middle line, which runs forward from the anterior border of the foramen to the extreme anterior end where it is truncated abruptly for a cartilaginous extension. There seems no doubt that this ridge gave attachment to a membranous inter-orbital septum which must have risen for a considerable distance before it divided to surround the anterior end of the brain. The transverse flanges are shown to be very deep and to bear articular facets facing directly anteriorly, with which a special process of the anterior bone of the palato-quadrate cartilage articulated. Mesially of these facets the dorsal surface of the bone bears a pair of grooves which begin at shallow notches on the lateral borders of the ventral surface of the bone and pass upward and toward the mid-line behind the border of the foramen. These grooves were presumably associated with the internal carotid arteries. They end at a margin which maps out a triangular area, within which lies a small apparently independent bone with no features other than a small circular pit which passes down into its substance and ends in a rounded surface. Behind this bone and lateral to it the transverse flanges are thin and their upper surface featureless. When seen from its dorsal surface the anterior basi-cranial element is wide anteriorly, but ventrally it is hidden by four independent ossicles—a smaller pair lying adjacent to one another and immediately anterior to the pointed front end of the basi-cranial bone, and a larger pair which lie laterally and are indeed independent because one of them is slightly misplaced in the specimen. Finally the whole structure ends with a very thin transversely expanded plate of bone which crosses and lies in contact with the anterior margin of these four small elements.

The dorsal surface of the temporal region of the brain case is formed in large part by a pair of very big bones which may in extremely old individuals have become co-ossified across the middle line, but which in younger though still large specimens were certainly separate. Anteriorly they remain distinct, even in my isolated neural cranium. At the extreme anterior end each forms a narrow and sharp point, lying in the orbital margin, whose inner border seems to be continued directly backward parallel to the mid-line perhaps throughout the entire length of the bone. On its dorsal surface the bone forms a convex surface which descends laterally and extends out to the almost transversely placed orbital margin which ends abruptly at a massive post-orbital process. The hinder wall of the orbit is in part ossified as a spheroidal

surface extending down from this margin; it is shown to be perforated by a rather small canal passing directly backward from a point some distance below the dorsal surface and mesially of the post-orbital process. Immediately below this foramen the bone forms a very massive pillar nearly circular in horizontal section, separating the orbit from a deep depression in the otic region within which lies the facet for the articulation of the otic process of the palato-quadrato. It is perfectly clear that this column was formerly connected by cartilage with the summit of the transverse ramus of the anterior basi-cranial bone. It is possible to determine the distance which separated these two bony surfaces by continuing the transverse rami dorsally until they become so far separated that they meet the proper spots on the dorsal bones; the height so determined can be checked because the depth of the palato-quadrato between the articulation of its otic process with the dorsal bone, and its basi-cranial articulation, is known. Both methods give the same height for the neural cranium in this region, and the diameter of the eye, determinable from the orbital plates surrounding it, is entirely appropriate to the orbital cavity so reconstructed. The lateral surface of the dorsal bone immediately above this descending column is concave, but soon shows a well-defined convexity marking the outer end of a concave face on its under surface, which is clearly the point of articulation of the otic process of the palato-quadrato. Posteriorly to the wide ventral expansion the bone is only represented by the ventral edge of its thin lateral surface, which stands more or less vertically in the skull. The dorsal surface of the bone in this region seems to be a direct continuation of that overhanging the orbit, and the bone terminates at a transversely placed margin. Trial with a plasticene model and general probability suggest that in the region immediately behind the column descending from the dorsal bone, the cartilaginous side wall of the brain case stood almost exactly vertically, but inclined a little outwards toward the dorsal surface. It seems to be certain that anteriorly it formed a deeply overhung depression like the corresponding region in a Palaeoniscid brain case.

One additional pair of elements lies posteriorly, each of which is a squarish bone now displaced in the specimen but apparently lying in the side wall of the occipital region. The anterior margin is a thick smoothly rounded column, with a well-defined notch toward its lower end below which the bone is produced to a point. The inner surface of the bone here is smooth and forms part of a cylinder with a horizontal axis. Unquestionably it formed part of the side wall of the actual brain cavity. The outer surface behind the anterior margin is at its upper edge produced into a short outstanding process which must have been in contact, or nearly so, with the cranial roof. Behind and below this process the outer surface bears a shallow groove which forms a quadrant extending from the posterior upper corner of the bone toward the posterior lower corner. This groove at about one-third of its length from the ventral end is entered by a small foramen, which is shown to pass through into the cranial cavity. Still more posteriorly but at the same general level there is another small foramen, also shown on both surfaces, which itself opens into the lower end of a much more shallow

groove passing dorsally and appearing to join that just described. It seems certain that the antero-ventral corner of this bone was in contact with the lateral flange of the anterior end of the posterior basi-cranial bone, and that the whole lay nearly vertically but inclined somewhat outward dorsally, in the side wall of the occipital region. If this were so then the notch in the anterior border must have been for the vagus nerve, and the two posteriorly placed foramina for spinal-occipital nerves whose dorsal rami passed up the grooves, the ventral ramus of the more anterior lying in the ventral part of its groove. The posteriorly and laterally directed foramen, which seems to have existed between the ventral border of the posterior basi-cranial bone and this lateral-occipital element, remains without explanation. I am acquainted with nothing in fish which will account for it, although it is conceivable, and no more, that the jugular vein comes out through it. An apparently homologous foramen in the Arthrodeir *Leiosteus* is believed by STENSIO to have transmitted the radix aortae.

Several specimens show that the first ossifications to appear in the neural cranium are cylinders of bone surrounding the anterior and posterior vertical semi-circular canals. These were first figured and recognized by BASHFORD DEAN (1907, fig. 29). In D.M.S.W. P. 470 these are in contact by their upper ends and do not extend down to the ampullae; there is no trace of utriculus nor of the horizontal canal. An Edinburgh specimen shows an extension of ossification to surround the ampullae of the vertical canals and to enclose part of the horizontal semicircular canal. There is still no sign of the utriculus or sacculus. The only other ossification in the neural cranium of this specimen is in the anterior part (to the hinder border of the foramen) of the anterior basi-cranial bone. These specimens show the position and size of the ear which lies, as might be expected, in the side wall of the neural cranium above the middle basi-cranial bone. The general structure of the semicircular canals, their length and slenderness, is comparable with that of the Elasmobranchs, and stands in marked contrast to that of the corresponding structures in Osteolepis and Palaeoniscids. There is, however, no visible trace of the complexities which are commonly found in modern Elasmobranch labyrinths.

Visceral Skeleton—As REIS originally discovered and as JAEKEL and BASHFORD DEAN have confirmed, the palato-quadrate cartilage in *Acanthodes bronni* is ossified as three independent structures. It is quite clear from the character of the material that these bones during life surrounded parts of a continuous cartilaginous palato-quadrate. The whole palato-quadrate cartilage so restored resembles that of an Elasmobranch in its general character except for the wide separation of the anterior end from that of its fellow. It has a very short palatal portion and a greatly elongated deep paraotic region whose dorsal margin is everted so as to form a thick and powerful ridge projecting laterally above the recessed surface of the rest of the bone. This ridge becomes somewhat deeper as it is traced posteriorly and ultimately terminates at the quadrate condyle, which lies a little obliquely to the general length of the bone and is

comparatively narrow from side to side. The hinder half of the cartilage is sheathed by a single bone, whose lower margin for the anterior half of its length is turned outward, so that the structure forms a nearly horizontal surface on the palate. Behind this there is a well-marked semi-elliptical notch for the masticatory muscle. The dorsal element, which bears the otic process and unquestionably articulated with the dorsal bone of the neural cranium, has a marked vertical ridge along its anterior margin which lies immediately behind that post-orbital bar of the neural cranium whose lower end is the transverse flange of the anterior basi-cranial bone. The bone is perforated by a foramen which passes dorsally through its anterior and upper corner; the significance of this foramen is obscure. The anterior bone of the palato-quadrates forms a shallowly concave shell which continues the everted anterior end of the posterior bone and lies at a low angle with the horizontal in the palate. The bone terminates anteriorly in a point lying under the middle of the orbit. From this point backwards the inner edge is gently concave until it ends abruptly at a backward-facing facet, which is shown in several specimens to articulate with the front face of the transverse flange of the anterior basi-cranial bone. Behind this facet the bone is cut out into a deep rounded notch and then comes into contact with the dorsal element in the palato-quadrates, with which it may in old individuals be continuous. The upper jaw is thus well supported by two articulations with the neural cranium lying almost in the same transverse plane. It is certain that the palato-quadrates of the two sides did not meet in the middle line anteriorly as they do in Elasmobranchs and in the sturgeon.

The lower jaw is ossified as two independent bones which, even in the largest specimens, were separated by a considerable mass of cartilage. The whole structure has much the appearance of Meckel's cartilage of an Elasmobranch. The lower margin of the hinder half is turned out to form an outstanding ridge which posteriorly passes upward to form the labial side of the articular region, the latter bearing a concave facet for articulation with the quadrate. Posteriorly the structure is thin from side to side, but anteriorly it widens so that the oral surface is nearly flat and it is roughly triangular in section. It narrows very rapidly and becomes extremely slender anteriorly, where it is slightly turned inward and bears a very small pit, presumably for the short ligament by which it was attached to its fellow of the opposite side.

The structures so far described consist of a thin film of bone surrounding the still persistent cartilages, but the lower jaw includes also a structure, REIS's extra-mandibular spine, which is clearly of a different nature. It is completely formed even in such very small specimens as B.M.N.H. P. 6192, where there are no cartilage bones whatsoever in the head. The extra-mandibular spine is a massive and nearly straight splint firmly attached to the lower and outer surfaces of the lower jaw; it lies indeed in a groove in that structure and extends continuously from a point near its anterior end to the middle of the articular surface. The morphological significance of this structure is uncertain but it clearly belongs to the mandibular arch. As REIS has already recognized it is associated with a series of delicate bony rods, called by him the extra-

mandibular rays. Many specimens (that in *Pollichia* being especially useful) show that these rays are definitely related to the mandible and retain their positions even where the hyoid arch is misplaced. Young individuals as a whole confirm this arrangement. The rays indeed seem to have been attached to the lower surface of the extra-mandibular spine and are constantly seen crossing it in many specimens. They begin with one or two pairs which are short, lie parallel to the middle line, and are far removed from the lower jaws. These are followed by about twenty more of which the anterior are S-shaped bones, the outer ends tending to lie parallel to the middle line, the middle portion transversely, and the inner ends being directed backward (B.M.N.H. 22658 has twenty-four pairs). Posteriorly these rods become straighter and project backward as a fringe from the outer surface of the hinder end of the mandible; towards the articulation of the upper and lower jaws the attached ends of these bones are turned downward. A sudden break occurs at the articulation, the three or four rays lodged on the posterior edge of the palato-quadrate having an upturned articular end. A comparison of this account with that which I have given for the opercular rays of *Mesacanthus* and *Cheiracanthus* will show that it is beyond question that the extra-mandibular rays in *Acanthodes* lay in the operculum, the greater part of which was unsupported by skeletal structures and not even protected by a scaly covering, its existence and extent being shown only by the lateral line crossing it. It is, I think, abundantly clear from the whole character of this material, and especially from those specimens in which the hyoid arch, as represented by its continuous series of gill-rakers, has undergone some displacement, that the whole opercular skeleton was supported by the lower jaw and the palato-quadrate cartilage and had no connexion with the hyoid arch. This series of opercular rays is found early in development; nineteen pairs are present in P. 6192.

The hyoid arch is very well displayed in many specimens, especially *Pollichia* and P. 323, P. 493 and P. 494 in my own collection. It is angulated, forming a horizontally placed V, the joint along which it was movable lying in the same horizontal plane as the articulation of the lower jaw, though its articulation lay between residual cartilages, the bones (epi-hyal and cerato-hyal) not nearly reaching one another. The epi-hyal or hyomandibular lies mesially of the posterior bone in the palato-quadrate when in its natural position. Its widened upper end lies almost on the same level as the upper margin of that bone but the narrower lower extremity projects some distance behind it. It is clear that the upper end of the bone lies high in the head and might be expected to articulate with the dorsal bone of the neural cranium, which, however, shows no sign whatsoever of any facet for such a contact. REIS, JAEKEL and BASHFORD DEAN are in agreement that there is another element in the hyoid arch which would represent a pharyngo-hyal and lie dorsally to the epi-hyal. None of the materials at my disposal show a trace of such a bone and some of these are so well preserved and satisfactorily displayed as to make me doubtful of its actual existence, although in face of the consensus of opinion of the authors I have mentioned I should be very sorry to deny its presence. The ventral

portion of the hyoid arch is formed by two bones, the cerato- and hypo-hyals, which meet one another. At their point of contact each is very deep dorso-ventrally and each is reduced in height as it leaves this region until both anteriorly and posteriorly the structure is quite shallow. The posterior bone is nearly straight, the anterior turned a little inward toward the middle line and ending in an enlarged knob for articulation with the basi-hyal. The ventral surface of the two bones in the middle of their length bears a shallow longitudinal groove which passes out on to the lateral surface posteriorly and dies away anteriorly. The basi-hyal is a T-shaped bone, the hypo-hyals articulating in the angles between the leg and the cross-stroke. It possesses a short not very deep posterior extension in the middle line, and was in all probability in contact with the first of a series of cartilaginous basi-branchials, of whose existence my material gives no satisfactory evidence. The complete hyoid arch, from the dorsal end of the epi-hyal to the basi-hyal, supports a series of gill-rakers of very characteristic and remarkable structure. Each one consists of a blade, relatively wide from side to side, widening greatly at the middle of its length but extremely narrow from back to front. It has a lenticular transverse section and its free extremity is pointed. The surface bears a series of irregular low longitudinal ridges and furrows which lie in the main parallel to its length. The blade of the gill-raker is a little narrowed just above its base. The base itself is a hollow bone expanded in a plane at right angles to the breadth of the whole element. It is thick so that its cavity is nearly circular in section. The attached surface, which lies in very close contact with the underlying bone of the hyoid arch, is a hollow half-cylinder placed transversely to the length of the hyoid arch. In consequence the visceral surfaces of all the bones of the hyoid and branchial arches which bear gill-rakers are crossed by low ridges on to which the bases of the gill-rakers fit. The dorsal part of the hyoid arch bears about thirteen gill-rakers, the ventral part about thirty-two in a large specimen. The whole arrangement of the gill-rakers on the ventral portion of the hyoid arch is exceptionally well shown in a specimen of the Coal Measure, *Acanthodes wardi* from the Knowles Ironstone, which is number LL. 181 in the Manchester Museum. Here it is shown that anteriorly the gill-rakers of the two sides come together so that their bases were inserted side by side, presumably on the oral surface of the basi-hyoid bone. Whether this arrangement occurs in precisely the same form in *A. bronni* is uncertain. In young individuals at least it apparently did not, the series of gill-rakers terminating whilst the hyoid arches are still separated. There is, however, in the *Pollichia* specimen evidence of certain small structures seen only in broken section which have not the appearance of bone but rather that of gill-rakers. They lie laterally to, and also immediately in front of the wide anterior end of the basi-hyoid bone. It is thus clear that the whole hyoid arch, from end to end, was coated with a single series of extremely powerful bony spikes. In young specimens the gill-rakers are only developed over the hypo- and cerato-hyals, those supported by the epi-hyal only appearing after ossification in the palato-quadrato is well advanced.

The relationship of the hyoid to the mandibular arch is difficult to establish. In certain cases, the *Pollichia* specimen for example, where the palato-quadrates have been rotated outward it has carried the epi-hyal with it, an indication of a close relationship and some attachment by soft tissues. But in other cases, the original of REIS's 1895, Plate V for example, where all the visceral arches have been dragged sideways and have rotated about the articulations of the palato-quadrates with the neural cranium, the hyoid arch has been separated from the mandibular arch and now leaves a space about as great as that separating the hyoid from the first branchial arch. In the specimen P. 495, which shows the neural cranium, the epi-hyal, pharyngo-branchial and epi-branchial of the first branchial arch have almost retained their natural relationship to one another, although they are scattered and no trace of the mandibular arch remains. It seems therefore that the attachment of the hyoid arch to the mandibular arch was no closer than its association with the first branchial.

The first structures associated with the branchial arches to be calcified are the gill-rakers. These first appear at the point of angulation of each arch between the epi- and cerato-branchial, and form a short series with a very characteristic festoon appearance, the attached bases of the denticles making a narrow U. It seems certain that the development of these structures began on the first arch, the others following in turn, the gill-rakers on the fifth arch only appearing in really large fish in which the ossification of the mandibular arch is complete.

The fact of the existence of so many different species in Lebach makes it impossible to trace the course of this calcification in detail. When fully developed in the largest heads, e.g. D.M.S.W. P. 323, and *Pollichia* II, the first branchial arch contains four bones. The pharyngo-branchial is a bone of characteristic shape which, in contrast to the equivalent cartilage in all Elasmobranchs, is directed forward, its ventral border continuing the curve of the epi-branchial. Its upper border has a small roughened projection, relatively thin from side to side. The posterior end of this bone in the largest specimens approaches the anterior end of the epi-branchial, which is a laterally flattened cylinder of bone. The angulation of the arch lies in the wide space between the epi- and cerato-branchials. The laterally flattened cerato-branchial is a bone of considerable length whose anterior end lies about in the plane of the hinder end of the lower jaw. The arch then contains a long slender hypo-branchial bone which directly continues the line of the cerato-branchial and anteriorly ends in a slender rounded point behind which its admesial border forms a low process associated with the attachment to the unossified basi-branchial. In the largest specimens the whole of the inner surface of the first branchial arch, at least from the upper end of the epi-branchial to the anterior extremity of the hypo-branchial, is covered by a series of gill-rakers identical in structure with those on the hyoid arch. In the small but exceptionally completely ossified P. 494 the dorsal part of this series is perfectly shown, the tips of the gill-rakers overlapping and resting upon the inner surface of the epi-hyal.

The second branchial arch seems to be identical in its general character with the first, but ossification begins later and seldom if ever becomes so complete.

The third arch has an ossified hypo-branchial in the *Pollichia* specimen, and has only doubtful traces of bone in other specimens. It possesses, however, a fully developed series of gill-rakers.

The fourth arch does not contain any bone in any specimen I have seen, though in the very large specimen figured by REIS 1895, Plate V, it seems to be well ossified. In normal specimens its series of gill-rakers is short.

Some specimens, the one from *Pollichia* and one in Edinburgh for example, show a short series of gill-rakers belonging to the fifth arch, but the majority of specimens do not. Probably the arch and a gill slit before it are constantly present, but the gill-rakers only develop in extreme old age.

Consideration of the whole series of specimens makes it evident that the gill-rakers of each arch guarded the gill slit before that arch and their tips overlapped the arch in front. The arrangement was in all likelihood comparable with that in *Polyodon* or a herring, the V-shaped arches being separated by very long gill slits each converted into a sieve by this gill-raker series, the whole allowing the animal to feed on very small food. It seems probable that *Acanthodes* had already developed long gill filaments projecting freely into the gill chamber below the operculum as in bony fishes, and it is certain that it cannot have possessed the ordinary Elasmobranch type of gill.

The structure of the hyoid arch with its very extensive series of gill-rakers is so similar to that of the first branchial arch as to make it certain that the gill slit in front of the hyoid arch was as long dorso-ventrally as the one behind it. Hence the operculum must have been entirely of mandibular origin and a spiracle of normal fish pattern not developed.

The Exoskeleton of the Head—The younger specimens of *Acanthodes* in which ossification of the visceral arches and neuro-cranium is either non-existent or slight show the exoskeleton of the head extremely well; the different species vary considerably in the extent to which it is developed. In all the body is covered incompletely with a continuous coat of normal small square scales, which may become extremely tiny toward the dorsal and ventral margins. In most individuals, e.g. D.M.S.W. P. 490 and P. 494, and Edinburgh unnumbered, these scales die out behind the shoulder girdle leaving a naked triangular area bounded by short forward extensions of squamation round the main and ventral lateral lines. In these fish there is a complete absence of normal squamation and of dermal bones in the head, except for the ring of circum-orbital bones and for the rows of enlarged scales bordering the lateral-line grooves and canals. In older and more fully ossified specimens, e.g. D.M.S.W. P. 493, the anterior parts of the lateral-line apparatus are actually very slender canals supported by rows of bony cylinders. In a rarer type of *Acanthodes* from Lebach, B.M.N.H. 22658, B.M.N.H. P. 4477, and B.M.N.H. 40049, the squamation extends forward on to the head,

passing into a continuous shield of very thin polygonal bones just as it does in the earlier Acanthodians, *Climatius*, *Mesacanthus* and *Diplacanthus*. This region lies entirely dorsal to the main lateral line and orbit and does not continue over the snout. These dermal bones differ entirely from normal scales in that they are excessively thin, with convex external and concave internal surfaces, whilst they are no larger than the ordinary body scales. REIS, 1896, fig. 2 is a good representative of this type.

The only individually recognizable dermal bones are thus those surrounding the orbit. On the justifiable assumption that the orbital margin was circular these can be restored to their natural position and then prove to form part of a sphere. The series usually includes five bones (the commonest number in Acanthodians in general) but sometimes only four are present. Each bone has a smooth concave inner surface and an ornamented convex outer aspect. The width of the individual bones in the ring varies considerably and it is not clear that the widest plate is always in the same position. Their ornamented surface when taken in connexion with the character of the obviously homologous bones in other Acanthodians shows conclusively that these plates are circum-orbitals, and not as it is natural to suppose sclerotics. The eyes are always large, sometimes very large, and lie quite anteriorly, the snout being no more than a rounded surface connecting the anterior circum-orbitals of the two sides. Its surface passes back smoothly into the flattened wide inter-orbital part of the dorsal surface, which itself extends backward into the occipital surface and the trunk.

The main lateral-line lies rather above the mid-line of the flank and passes forward above the gill chamber over the eye, dorsal to the circum-orbitals, at least to a point half-way down the front of the orbit. From the main lateral line a variable number of side branches pass dorsally toward the mid-dorsal line. Specimens figured by Troschel show five and four, and by BASHFORD DEAN, five of such incomplete commissures in the anterior part of the trunk behind the shoulder girdle. P. 494 shows two, and many other specimens give similar evidence. Anteriorly to the shoulder girdle D.M.S.W. 490 shows two short branches, one above the angulation of the fourth branchial arch, the next above that of the second arch. From a point immediately in front of this latter a long branch passes inward and forward at an angle of about 45° to the main canal until it closely approaches, and may have coalesced with, the admesial end of another branch which arises from the main canal at right angles at a point a little in front of the jaw articulation. From the main canal the infra-orbital canal arises at a point some distance behind the posterior border of the circum-orbital ring and extends downward and then forward below the circum-orbitals at least to the level of the centre of the orbit. A mainly horizontal canal (the quadrato-jugal canal) takes its origin from the infra-orbital a little below the mid-point of its height and can, with certain interruptions by other structures of the head, be traced backward to the hinder end of the jaw, where it joins a more or less vertical canal (the pre-opercular canal). This arises from the main canal at the level of the hinder end of the posterior vertical semicircular canal, and, though interrupted by being lost amongst gill-rakers, can be traced downward to its junction with

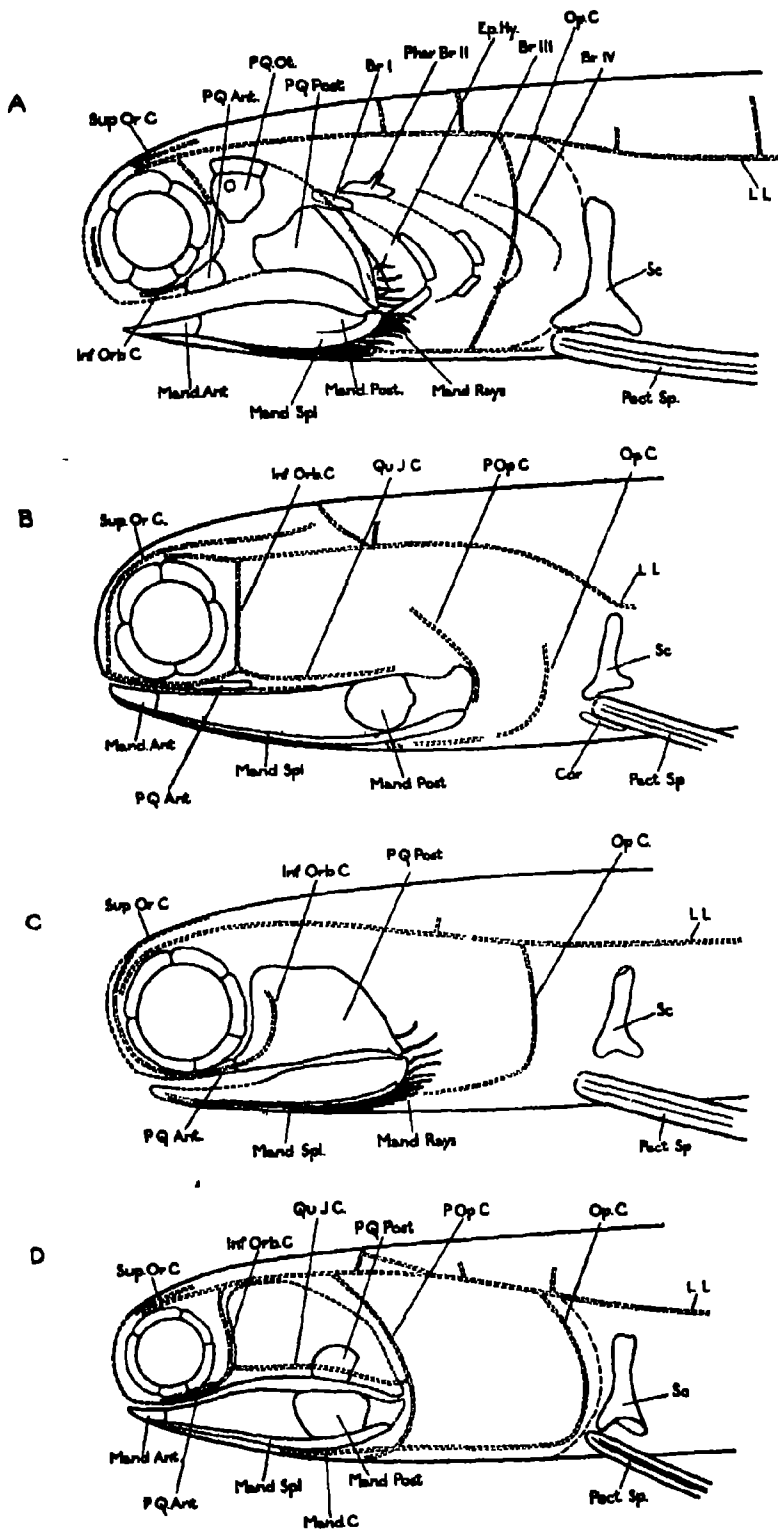


FIG. 20—*Acanthodes*. Partial reconstructions of the anterior ends of four individual specimens from the Lebach ironstones, various magnifications. A, D.M.S.W. P. 494 \times 1.05; B, B.M.N.H. 22658a and b \times 1.27; C, D.M.S.W. P. 498 \times 1.23; D, D.M.S.W. P. 496 \times 1.43. *Br. I*, *III*, *IV*, branchial arches, *I*, *III*, etc.; *Cor.* coracoid; *Ep.Hy.* epihyal; *Inf.Orb.C.* infra-orbital canal, of the lateralis system; *L.L.* main lateral-line canal; *Mand.Ant.* anterior ossification in Meckel's cartilage; *Mand.C.* mandibular canal; *Mand.Post.* posterior ossification in Meckel's cartilage; *Mand.Rays*, rays in the mandibular operculum; *Mand.Spl.* mandibular splint; *Op.C.* opercular canal of the lateralis system; *P.Op.C.* pre-opercular canal; *P.Q.Ant.* anterior bone in the palato-quadrates; *P.Q.Ot.* otic process of the palato-quadrates; *P.Q.Post.* posterior bone in the palato-quadrates; *Pect.Sp.* pectoral fin spine; *Phar.Br. II*, pharyngo-branchial *II*; *Qu.J.C.* quadrato-jugal canal; *Sc.* scapula; *Sup.Or.C.* supra-orbital canal.

the quadrato-jugal canal just above and behind the jaw articulation. The preopercular canal extends downward below this point, and its anterior prolongation is shown just ventral to the mandibular splint in the region anterior to the opercular rays. There is some evidence that the preopercular canal actually extends into the intergular space.

The main canal gives off one further ventral branch. The origin of this is not shown but its course is nearly vertical, parallel to the posterior margin of the gill chamber as far as the level of the ventral border of the lower jaw. It then turns abruptly and extends forward apparently to join the preopercular canal and form the canal of the lower jaw. The vertical part of this canal crosses the festoon of gill-rakers of the fourth branchial arch unaffected by them. It is thus evident that it was carried on the operculum. The presence of this opercular canal is confirmed by several other specimens, e.g. D.M.S.W. P. 498, and P. 494, where it crosses between the angulations of the second and third arches without conforming to them. An unnumbered specimen from Berlin is important because it is the only one in addition to P. 490 which shows with perfect clearness the infra-orbital, preopercular and opercular lateral-line canals.

The supra-orbital canal in D.M.S.W. P. 490 begins at the level of the front of the ear and extends forward parallel to, and mesial of the main canal until it turns down on to the snout in front of the orbit, its further course being incapable of determination. Consideration of the whole material seems to show that the infra-orbital canal passes round the eye until it terminates dorsal to the orbit, not far in front of the anterior end of the main lateral line. A branch arising from this canal passes downward and inward over the snout, turning up again to meet the anterior end of the supra-orbital canal, which at this point is connected to its fellow by a V-shaped commissure. It is unfortunately impossible to be certain of these connexions, which indeed may vary a little from specimen to specimen. B.M.N.H. P. 4477 is important because Miss TOWNEND found in it a circular space, free from all trace of bone, and in part bounded by a definite small plate which may plausibly be interpreted as a nostril. This lies near the middle line, dorsal to the lateral-line commissure, and mesial of the supra-orbital canal.

Body and Fins—The contorted position of most specimens of *Acanthodes* from LEBACH makes it difficult to draw accurate reconstructions of the whole fish, but in some cases it is possible by following the main or the ventral lateral lines to determine the proportions with reasonable certainty. It is, however, unfortunate that the tail is almost always missing, though the anterior margin of its ventral lobe is often included in the nodules. In Table II I give the head length, depth of body and length to the dorsal fin in mm. for a series of specimens, determined from restorations so made.

The general shape of *Acanthodes* was quite well illustrated by ROEMER. It is a very slender eel-like fish with a heterocercal tail, whose hypocaudal lobe is shorter than the extension of the body and projects downward. The single dorsal and the anal fin are placed very far back, the anal being larger than the dorsal and a little in front of it. The pectoral fins are "enormous" and the pelvics small.

TABLE II

	Head length	Depth	Length to dorsal	Length Head length	Length Depth
	mm.	mm.	mm.	mm.	mm.
B.M.N.H. P. 6192	20	12	76	3.8	6.3
40050	31	14	131	4.2	9.3
D.M.S.W. P. 490	49	32	198	4.0	6.2
P. 498	58	31	213	3.7	6.8
P. 494	69	39	305	4.4	7.7
B.M.N.H. 40049	100	—	440?	—	6.6

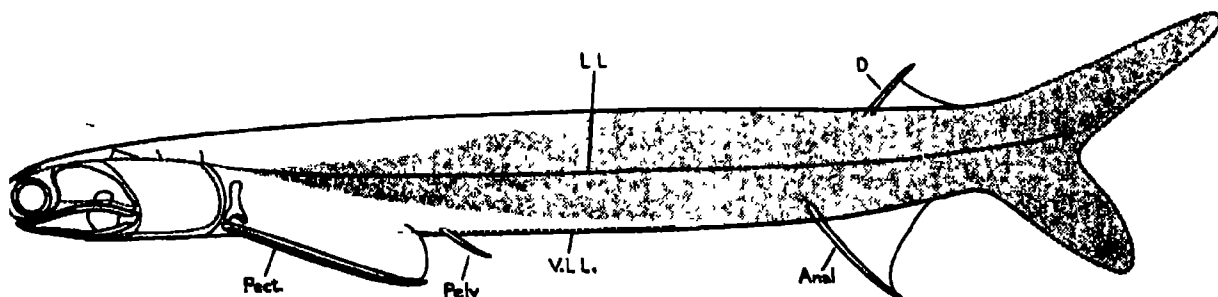


FIG. 21.—*Acanthodes* sp. Lebach ironstone. Reconstruction of specimen P. 496, D.M.S.W. collection. $\times 0.6$. The area covered with mechanical tint represents the extent of the squamation. *Anal.* anal fin; *D.* dorsal fin; *L.L.* main lateral-line; *Pect.* pectoral fin; *Pelv.* pelvic fin; *V.L.L.* ventral lateral-line.

The development of scales on the body can be determined in outline. The first scales to appear are those which border the main lateral-line canal in the hinder part of the body, new scales being added both above and below the lateral line and at its anterior end. In B.M.N.H. P. 6192 (76.0 mm. long) both lobes of the caudal fin are completely scaled, though the fin is here much more deeply cleft than it would ultimately become. That part of the body behind the median fins is covered with scales throughout its height, though the dorsal and ventral surfaces seem to be scaleless. There are no scales on the fin webs. In front of the median fins the squamation covers a decreasing depth of the flank, the lateral-line scales alone remaining at a point about half-way between the pectoral and anal fins, and no traces of lateral-line bones being visible in the anterior part of the trunk or on the head. In B.M.N.H. 40050 the main lateral-line can be traced forward on to the head, and the patch of flank scales associated with it has extended farther forward and posteriorly is deeper. In addition a new ventral strip of squamation has appeared. This is associated with the pair of ventral lateral-line canals which arise quite anteriorly between the pectoral fins and extend backward to the anal fin. In the anterior part of their course the two lines are separated by a small space, the width of about six scales, but posteriorly they come together, the admesial edges of the ventral rows of enlarged scales bounding them being in contact or nearly so. This arrangement was well figured by TROSCHEL (1857, Taf. I, figs. 1 and 3), but has escaped the notice of all subsequent writers. The ventral lateral-line area of

scales is certainly discontinuous with the flank series from its anterior end as far back as the anal fin, but appears to join them in that region, an indication that the hinder part of the body was already completely scaled. No scales occur on the webs of any of the fins other than the caudal.

In larger specimens, e.g. D.M.S.W. P. 490, the spread of the scaled areas continues, the mid-ventral strip remaining separate and a long triangular area behind the shoulder girdle being still free from scales. The main trunk scaling now continues forward as a slender triangular area to the region of the shoulder girdle. The body seems to have been essentially completely scaled behind a point about one-third of the distance from the dorsal fin to the pectoral fins. In this fish a considerable area of the base of the web of the anal fin and a small part of that of the dorsal fin are scale covered, but the pelvic and pectoral fins are scaleless. In B.M.N.H. 40049, the anterior third of a large fish with a lower jaw length of 6.6 cm. and a total length to the dorsal fin of presumably about 450 mm., the scales continue uninterruptedly over the dorsal surface and extend forward to the ear. A scanty ventral strip of scales can be traced to the hinder end of the lower jaw, but the triangular area behind the shoulder girdle still remains free from scales. The size of the scales is on the whole similar over the whole flank of a fish, but they decrease a little toward the dorsal and ventral surfaces. The scales on the fin webs are often extremely small. This mode of development of the squamation gives the explanation of the remarkable fact that the scales of large specimens of *Acanthodes* may be no larger than those of small individuals. The scales of the specimens last considered are of the same size within the necessarily wide limits of measurement. Those of the Edinburgh specimen, about 230 mm. in length, are about twice as wide as those of the series above described.

The caudal fin is very seldom well preserved; that of a small specimen belonging to Berlin is illustrated in fig. 6, Plate 13. In this the interesting features are the extension of the main lateral line to a termination just in front of the fork of the tail and the absence of any upturning of its posterior end; the presence of a narrow mid-dorsal strip of scales with a free posterior end identical with a similar arrangement in *Euthacanthus* and *Mesacanthus*; and the occurrence of a sharply differentiated strip of scaling parallel to the dorsal margin of the upper lobe of the fin, again a parallel to the two genera last mentioned. The lower lobe has a rounded termination lacking the peak of certain genera. I can add nothing to the account and figures of the tails of full-grown specimens of "*Acanthodes bronni*" given by KNER (1868, pl. V, fig. 2, and pl. VII, fig. 1). These specimens show the presence of ossified neural and haemal arches in the tail and of a series of long radials, extending about two-fifths of the distance to the tip of the ventral lobe, unjointed and each corresponding to a haemal arch.

The structure of the other fins of *Acanthodes* is best shown by specimen D.M.S.W. P. 498. Here the dorsal fin spine has the normal structure. It is a nearly straight rod with flattened lateral surfaces. The rounded anterior margin is marked off by a well-defined groove on each side of the spine. There is a cavity in the lower part of the spine

which is open toward the web of the fin, and the spine has an inserted base of considerable length. A single basal bone extending backward from the spine is indicated by a rather obscure impression. Dorsal to this bone is a series of delicate bony fin rays, very short and no doubt paired. Surrounding these and passing farther out into the web is a small patch of normal square scales.

The anal fin of this specimen, though larger, is similar to the dorsal fin. No trace of a basal can be seen nor are the fin rays visible, but a large area of the fin web is covered

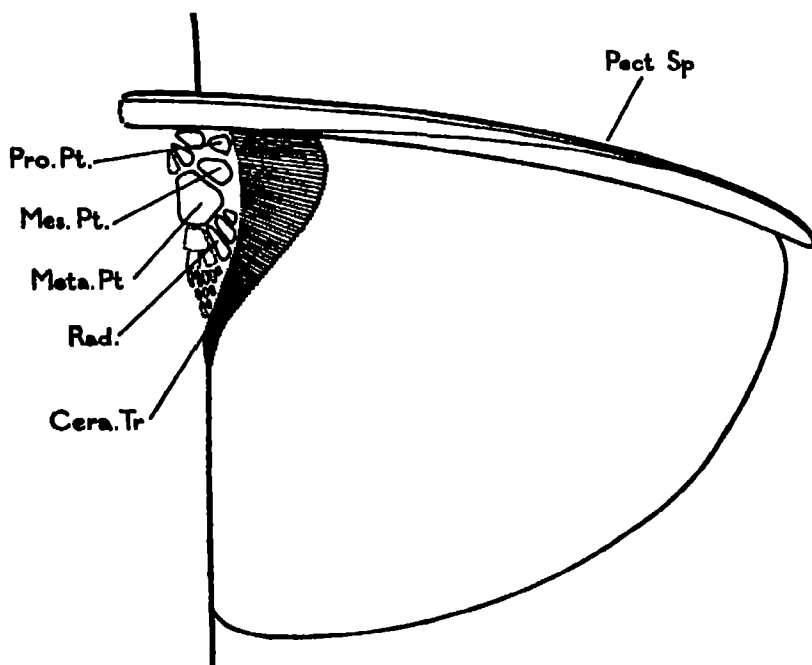


FIG. 22.—*Acanthodes* sp. Reconstruction of a pectoral fin, the skeleton from P. 498, and the outline from P. 490, D.M.S W. collection; both are from Lebach. $\times 1.6$. *Cera.Tr.* cerato-trichia; *Mes.Pt.* meso-pterygium; *Meta.Pt.* meta-pterygium; *Pect.Sp.* pectoral spine; *Pro.Pt.* propterygium; *Rad.* radials.

with square scales whose sides lie respectively parallel and at right angles to the ventral line of the body. These scales become smaller as they are traced toward the margin of the fin. In P. 490 the anal fin is represented by its spine, by a low scaled area extending far posteriorly toward the caudal fin, and by a carbonaceous film which is part of the delicate unscaled distal area of the web.

The pectoral fins are well shown in P. 498, in which the shoulder girdle has its normal structure. It is a bony cylinder, very nearly circular in transverse section and standing vertically in the body, its slightly expanded upper end reaching only to about half-way up the flank. The lower end is widened antero-posteriorly and is produced downward into lappets, that on the inner surface lying anteriorly, and that on the outer surface at the hinder end. The bone contains an hour-glass-shaped cavity. The

shape and character of this bone, apart from its evident relationship to the homologous structures in other Acanthodians, show that it was laid down round a cartilage, and I call this bone the scapula. No other bone is to be seen in the shoulder girdle in most specimens, but in B.M.N.H. 22658 and 40049 another small rounded but flattened bone lies ventral to the scapula. The homology of this bone is uncertain; it may be a cartilage bone, a coracoid, or it might be of ultimate dermal derivation. Its homologue is known in *Cheiracanthus*.

Even in large specimens the pectoral fin usually has no ossifications in its cartilage, but in D.M.S.W. P. 498 there are well-developed basal and radial elements. It is evident that these are radially arranged, the basals being concentrated. In the absence of any certainty as to the position of the articulation between the shoulder girdle and the fin skeleton it is not possible to be sure of the structure, but the most reasonable interpretation is to regard the fin as tribasal, of general Elasmobranch character. At any rate the proximal row represented consists of a short and small pro-pterygium supporting a single radial, a small meso-pterygial with a long and stout radial and a large meta-pterygial with a narrow base widening greatly as it is followed distally. This bone supports two slender radials which are in contact with one another, and extends admesially of them leaving space for further radials and probably for an extension of a meta-pterygial axis, of which no trace remains. The base of the fin web is supported by two series, dorsal and ventral, of short, massive, closely set dermatrichia. These are straight rods ossifying very early in the life of the animal. They form a graded series, the longest on the outer side of the fin just within the fin spine, those lying toward the inner margin being very short. The proximal ends of these rods lie on a smooth curve which sweeps inward from a point little removed from the shoulder girdle to one at a considerable distance from the base on the inner side of the fin. Distal to this series lies another, similar in general character but usually of more slender and less closely set rods. In the great majority of specimens these are short, but in D.M.S.W. P. 494 some of them are extraordinarily long, extending out nearly, but not quite, to the margin of the fin. The fin web is beautifully shown as a carbonaceous film in D.M.S.W. P. 490 and P. 494. The fin is of extraordinary width; the fin spines can be erected so that the two make an angle of 130° with one another (in P. 490), and the fins, though fully extended, are not then torn away from the body. The evidence of this specimen seems to show that the tip of the spine projected beyond the margin of the fin, and there is evidence of non-ossified dermatrichia reaching the fin border. In laterally compressed specimens the series of dermatrichia is usually folded on itself in such a way as to suggest that the inner margin of the fin was attached to the body near the ventral surface for a long distance. The whole fin is usually devoid of scales, but D.M.S.W. P. 321 shows a small patch of small square scales which become rounded and radially arranged distally, covering the region of the radials and proximal dermatrichia.

The pelvic fin is represented only by its spine in all specimens seen.

Professor KNER (1868, pl. V, fig. 2) figured a specimen of *Acanthodes* which contained a mass of *Estheria* in the region of the abdominal cavity. I have occasionally seen single specimens of *Estheria* within *Acanthodes*, and the crustacean is commonly seen in the ironstone nodules containing that animal. There is, however, in the Edinburgh Museum a specimen (1891, 16, 3) of *Acanthodes sulcatus* from the Straiton Ironstone (Lower Carboniferous) which contains within its body cavity the disorganized remains of a specimen of the Palaeoniscid fish *Cryphiolepis*. These remains occupy a length of some 12 cm., the *Acanthodes* has pectoral spines of about 5 cm. and its length from snout to anal fin may be estimated at 30 cm. Thus the food of *Acanthodes* was more varied than its toothless condition and elaborate filter apparatus of gill-rakers might suggest. But it is probable that it did in general live on small animals which, as in the case of *Estheria*, need not necessarily have been plankton.

ON THE NATURE OF THE HARD PARTS OF ACANTHODIANS

I have not made any extensive investigation of the histology of the bones and scales of Acanthodians because I am doubtful whether the materials from Turin Hill, which are the most abundant and interesting of those at my disposal, give a true picture of the structure. In the absence of sections of some animal from those rocks, known to be bony, it must remain uncertain whether the absence of cell spaces in the jaw bones and scales of *Ischnacanthus* is primary or results from unsuitable preservation.

I have never myself seen unquestionable lacunae in any Acanthodian material, but a section, which Professor A. KARPINSKY was kind enough to send me, through some very large scales presumably of *Acanthodes* from the Upper Devonian or Lower Carboniferous of the North Ural, shows very rarely bipolar spaces which may have contained bone cells. These lie scattered amongst the fine canals which penetrate the whole thick rounded root of the scale, below its "ganoine" surface, and in one case form a continuous though widely spaced layer. More generally only about half a dozen are to be seen in a section of a scale about 1 mm. sq. With this exception the structure of these scales is exactly as GOODRICH (1909, p. 188) describes it, the base of the scale being perforated by immense numbers of minute little branched canaliculi. I am thus very doubtful whether the scales described by BROTZEN (1934*a* and *b*) are all rightly referred to the Acanthodii. In any case I am sure that it is impossible to determine isolated Acanthodian scales generically.

The calcified material associated with the cartilaginous skeleton of Acanthodians has been examined in section by REIS (1896), SMITH WOODWARD (1917) and by HANCOCK and ATTHEY (1869). I have cut sections of jaw elements of *Ischnacanthus* and *Acanthodes*, and of the scapula of *Acanthodes*. SMITH WOODWARD describes the structure of a very large Acanthodian jaw (*Plectrodus*) as follows: "The hard base to which the teeth are affixed proves to consist of almost structureless translucent calcified tissue in which there are occasional streams of elongated cellular spaces, irregular in shape, and some-

times with traces of ramifying canaliculi. It thus agrees exactly with the corresponding tissue in *Ischnacanthus*." HANCOCK and ATTHEY (1869) say of *Acanthodopsis*: "The jaw itself is composed of very dense bone on the surface, in which the Haversian canals are well defined, and the radiating cells are very numerous and minute; they are elongated fusiform, with the canaliculi (when observable) sufficiently abundant, and arranged for the most part at right angles to the long axis of the cells. . . . In the superficial and denser portions of the tissue, the cells and tubules are the most minute; in the deeper portions they are larger and less regular in form and the bone becomes riddled with medullary cavities, until at length it is entirely reduced to a sort of cellular structure." It is evident that this account, founded on very well-preserved material, is comparable with REIS's description and fig. (1896, pl. VII, fig. 1) of the jaw of *Acanthodes*, which is derived from much less well-preserved material.

Taken together these descriptions leave no real doubt that true bone does occur in the visceral ossifications of Acanthodians. But if no bone cells could be observed or even if they had never existed, it is evident from the fact that some portions of them must be resorbed during the growth of the animal that the jaw bones of *A. bronni* agree with true bone and differ from all other vertebrate hard parts.

SUMMARY OF THE STRUCTURE OF ACANTHODIANS IN GENERAL

The Acanthodians are fish-like gnathostomes whose general build is fusiform, with a body which may be rather deep (*Cheiracanthus*) but which is generally slender, ultimately becoming eel-like (*Acanthodes*).

General Shape

Fins—There are one or two dorsal, an anal and a caudal fin. The tail is always heterocercal, the muscular upper lobe being only slightly turned up, whilst the lower lobe is triangular. There are no ridge scales associated with the tail, but the upper edge of the upper lobe may have a special squamation ending in a fine point, perhaps a relic of a former third dorsal fin. The anterior border of the ventral lobe may be strengthened by a paired row of enlarged scales which extend backward as a hook.

The other median fins are supported by an anterior fin spine. In primitive forms (*Brachyacanthus*) this is extremely wide from side to side and contains a great cavity opening backward to the fin web; it is indeed nothing more than a thin scale-like plate bent round the anterior margin of the fin. In consonance with this structure the spine is restricted to the skin, its proximal margin being joined by ordinary body scales. In later forms the fin spine becomes laterally flattened, its very narrow cavity opening only within the body, and gains a definite root inserted between the myotomes.

In the primitive forms there is a long series of paired fins, usually represented only by their anterior spines. There may be as many as seven pairs; of these the first, the pectoral fins, are the largest, the remainder increasing regularly in size to the last, the

pelvic fins. The fin spines of the paired fins agree exactly in their structure with those of the median fins of the same fish.

Head—The head is very variable in its proportions; the mouth may be very short (*Diplacanthus*), or very long (*Acanthodes*), or of any intermediate length. The orbits always lie very far forward, so that the snout is short, merely a rounded anterior surface connecting them. A nostril is very seldom visible, and in all known cases (*Ischnacanthus*, *Cheiracanthus* and *Acanthodes*) lies high up, well removed from the mouth, and not far from the middle line.

Squamation—The body is covered with a very characteristic coating of extremely small square thick ganoid scales which lie close packed in contact with one another. Except in *Brachyacanthus*, where there is a row of large mid-dorsal scutes behind the head, there are never any enlarged mid-dorsal or mid-ventral scales. The size of the scales varies somewhat over the body. In many forms, though not in all, the lateral lines are bordered by enlarged scales.

In *Mesacanthus* and *Acanthodes* it can be shown that the squamation begins round the main and ventral lateral lines in the posterior part of the body, new scales being added dorsally and ventrally, the scale-clad area gradually extending forward toward the shoulder. The scales seem to reach their maximum size early in the life of the fish and growth takes place by the addition of new scales. In many forms the cheeks and ventral parts of the head remain without any covering of bony elements.

Structure of Head—The neural cranium is known at all completely only in *Acanthodes*, but *Cheiracanthus* seems to agree with that genus. The skull is tropibasic, there being a narrow inter-orbital septum with a brain cavity contained within its dorsal part. No trace of the nasal capsules or ethmoidal region can be seen. The orbito-temporal region is floored by a median T-shaped bone, whose anteriorly directed stem ends at a series of five small independent bones. The widened hinder end of the bone lies at the back of the eye, and its limbs are directed upward and outward towards the post-orbital processes of the paired dorsal bones. The anterior faces of the transverse flanges of this bone bear articular facets for the palato-quadrates. The ventral surface of the stem of the T-shaped bone has a groove which at its hinder end becomes a foramen for a persistent hypophysial duct.

The upper border of the orbit is ossified and ends in an outstanding post-orbital process. Behind and a little within this is a massive column formerly connected to the transverse wings of the T-shaped bone by cartilage. This is perforated by a horizontal jugular canal and its posterior face has a facet for the otic process of the palato-quadrates. The skull is narrow in the lower part of the otic region but it widens suddenly to a thick horizontal flange (? over the horizontal canal) toward the dorsal surface of the head.

The ventral surface of the skull in the otic region is made by a thin, formless sheet of bone which connects the T-shaped bone with the "basi-occipital". This latter has

a widened anterior end and then becomes very narrow, its lateral surfaces bearing deep grooves, which lead to large foramina anteriorly. They rise posteriorly to support the exoccipitals. The ventral surface of the basi-occipital bears a groove presumably for the dorsal aorta. The anterior border of the exoccipital is notched for the vagus and the bone is pierced by two foramina for occipital nerves.

Visceral Arches—The mandibular arch forms the upper and lower jaws. The palato-quadrates has a deep vertical paraotic portion whose nearly vertical anterior border lies behind the orbit and descends to a short more nearly horizontal palatal part. The palato-quadrates of the two sides of the head do not meet in symphysis. In *Climatius* they seem to have no contact with the neural cranium. In *Mesacanthus*, *Cheiracanthus* and *Acanthodes* the summit of the paraotic flange bears a definite otic process which articulates with the skull behind the post-orbital process, and the palatal part of the bone has a special basal articulation. In *Climatius* and *Cheiracanthus* the whole palato-quadrates in the adult forms a single bone, which is perforated by a vacuity in the paraotic region in the latter. In *Mesacanthus*, *Ischnacanthus* and *Acanthodes* the palatal ramus is an independent ossification, and in *Acanthodes* the otic portion is also independent.

Meckel's cartilage is always longer than the palato-quadrates, so that its symphysis lies in front of the anterior end of the palatal process. In *Climatius* and *Cheiracanthus* the lower jaw is in adults a single bone. In *Mesacanthus*, *Ischnacanthus* and *Acanthodes* it ossifies in two parts, an anterior and a posterior.

Teeth are present in the lower jaws of *Climatius* and *Parexus*; each tooth has a flat plate-like lower portion whose upper edge is cleft into small sharp-pointed cusps, usually a central large cusp and one or two smaller cusps both before and behind it. Three or four teeth of a single family are present simultaneously, forming a whorl. In *Ischnacanthus* there is a symphysial whorl of large simple teeth, and the palato-quadrates and Meckel's cartilage support a remarkable dentition of large and small teeth ankylosed to the bone, and a granulation on the lingual surface. In *Ischnacanthus* the anterior part of the buccal cavity is beset with a multitude of minute tricuspidate denticles. All other Acanthodians seem to be devoid of teeth.

The mandibular arch supports an operculum which stretches backward from the whole of the posterior border over the other visceral arches. In *Climatius*, *Parexus* and *Brachyacanthus* the greater part of this operculum, both between the lower jaws and also the whole periphery, is strengthened by a series of very small oat-like scales, but that part of the structure lying behind the articulation of the jaws contains a series of long opercular rays which merge into the scales covering the cheek. This operculum meets the ventral part of the shoulder girdle but does not cover the whole of the gill chamber. In *Euthacanthus* the series of opercular rays is much larger, extending downward to the middle line between the lower jaws; it is, however, short, covering only a small part of the side of the gill chamber. In *Mesacanthus* the series becomes

even more extensive, and the attached ends of the rays round the jaw articulation have a very characteristic structure. In *Cheiracanthus* the series of opercular rays reaches its maximum size, but retains the structure it has in *Mesacanthus* and still leaves part of the gill chamber uncovered. Finally in *Acanthodes* the opercular rays whilst still retaining their structure become reduced to very delicate short rods, the great operculum covering the entire gill chamber possessing only double rows of scales surrounding lateral-line canals. *Ischnacanthus* has a complete operculum covered by small scales but retaining small irregular series of rays, and *Diplacanthus*, which also seems to have a complete operculum, has a single very large bone in its proximal region.

Hyoid Arch—The hyoid arch cartilages are seldom ossified, but dermal ossifications are often to be found associated with it. The hyoid arch in Acanthodians is separated from the mandibular arch by a greatly elongated gill slit, as long as that which lies between the hyoid and first branchial arches. In all forms the hyoid arch is sharply bent, a ventral moiety articulating with a dorsal part at the level of the jaw articulation. In *Climatius* and *Cheiracanthus* the ventral part is composed of a single bone articulating in front with a median basi-hyal, whilst the dorsal part is unossified. In *Acanthodes* the dorsal part of the hyoid cartilage is surrounded by a single epi-hyal bone, perhaps developing an ossified pharyngo-hyal late in life. The ventral part is formed by well-ossified cerato- and hypo-hyals, the latter articulating with an ossified basi-hyal. In *Climatius*, *Brachyacanthus* and *Euthacanthus* the upper end of the hyoid arch has a series of longitudinal dermal bony splints. From these a hyoidean operculum extends backward to rest on the first branchial arch. The lower part of the arch is concealed by the mandibular arch and its operculum. In *Mesacanthus* similar dermal splints protect the upper edge of the hyoid arch, the operculum not containing any ossifications in this region, although on the level of the jaw articulation it possesses a series of short opercular rays, the serial homologues of those in the mandibular operculum. In *Cheiracanthus* the upper end of the hyoid arch is marked by two hook-like scales, but no dermal ossifications occur in connexion with it in *Ischnacanthus* or *Diplacanthus*. In *Acanthodes* the hyoid arch has no external dermal bones, but the visceral side of the whole structure is beset with a single series of very massive, close-set gill-rakers.

Branchial Arches—Four branchial arches of similar structure occur in *Climatius*. In them the skin contains a series of bony splints, lying parallel to the length of the arch, whose lower extremities are turned backward into an operculum the distal part of which is supported by small lineal splints lying horizontally. The series of opercula so formed overlap one another and cease, in the sense that they contain no more bones, at the free margin of the mandibular operculum. *Brachyacanthus* and *Euthacanthus* have an essentially similar structure, but show only three branchial arches. *Mesacanthus* shows a series of three or four curved opercular rodlets in each of its three branchial arches. In *Cheiracanthus* only longitudinally arranged splints without any sign of

opercular projection can be seen. In *Ischnacanthus* such longitudinal splints extend down to the level of the jaw articulation within the complete mandibular operculum. In *Acanthodes* there are no external dermal bones associated with the branchial arches, but the cartilages themselves are ossified. The first branchial arch in adults consists of four bones, a pharyngo-branchial directed forward continuing the course of the epi-branchial, which extends downward nearly to the point of sharp angulation of the arch. The ventral part of the arch contains a cerato- and a hypo-branchial bone, the latter reaching a basi-branchial. The whole inner surface of the arch bears a single series of gill-rakers identical with that on the hyoid. The posterior arches only differ in that ossification begins in them progressively later and seldom goes so far. Four branchial arches can usually be seen, a fifth rarely.

The Acanthodians thus form a series showing how the mandibular operculum, at first merely the largest of a series (one from the anterior margin of each gill slit), gradually extends backward until it covers the whole gill region, the smaller posterior opercula becoming functionless and disappearing *pari passu* with its development. It is evident that ultimately a Teleostome-like arrangement of V-shaped gill arches, separated by extremely long gill slits and bearing long gill filaments projecting outward into a gill chamber covered by an operculum, is achieved.

Lateral-Line System—A large part of the lateral-line system of Acanthodians remains in the condition of pit lines, although in the anterior part of the head some portion of the system sank down into true canals connected with the surface by small "primary" pores. The character of the material and nature of the system make it difficult to work out the distribution completely; the accounts given in this paper are incomplete but I hope accurate.

The body of all Acanthodians is traversed for a long distance (usually from the point of upturning of the ventral border of the muscular lobe of the tail) by a main (middle) lateral-line, which passes forward along the upper border of the gill chamber to the dorsal surface of the head. It extends onward in an essentially straight course above the orbit in *Ischnacanthus* and *Acanthodes* to, or nearly to, the level of the anterior edge of the orbital margin.

From this canal behind the head a very varied series of dorsally directed (commissural) branches pass upward toward the mid-dorsal line, but seldom reach it. In *Acanthodes*, two in my material, four or more in figures by TROSCHEL and BASHFORD DEAN, of these branches may lie behind the shoulder girdle. In *Diplacanthus* a single commissure lies immediately in front of the dorsal fin spine; the commissural branches which lie in the head region in front of the shoulder girdle are most variable. In *Euthacanthus* just posterior to the upper end of the hyoid arch there is a branch, inclined forward at 45° to the middle line, whose anterior end nearly joins a short transverse groove passing out toward the main canal. In *Mesacanthus* the apparently homologous grooves are directed forward and backward at 45°. In *Diplacanthus* the

anterior groove is transverse and the posterior absent. In *Ischnacanthus* there are three transverse grooves, the anterior joining the hinder end of a longitudinal canal. In *Acanthodes* the number of grooves visible ranges from two to four, one of the anterior members of the series often inclining inward and forward at 45°.

It seems impossible to homologize these canals individually through the series, but they clearly form a group from which the middle pit line and occipital commissure of ordinary fish could be derived.

The supra-orbital canal is never continuous with the main canal. It usually begins well behind the orbit and runs forward parallel to the main canal, ultimately passing down on to the rounded snout in front of the eye and apparently becoming continuous with the anterior end of the infra-orbital canal. In *Diplacanthus* the supra-orbital canals arise together in the mid-dorsal line far behind the orbit, and a pair of canals, starting from a spot just behind this point, pass outward and forward to join the main canal above the orbit in front of the point of departure of the infra-orbital canal. No homologue of these canals occurs in any normal fish, but they are exactly paralleled in *Arthrodeirs*. There is evidence of an ethmoidal cross-commissure in *Ischnacanthus* and *Acanthodes*, and it is probably of general occurrence.

The infra-orbital canal has its usual course round the orbit and seems to have been continuous with the supra-orbital canal. In *Euthacanthus* two posteriorly running canals arise from the infra-orbital canal behind the eye. The more ventral extends backward toward the jaw articulation, parallel to the mouth. It is the oral canal, the homologue of the quadrato-jugal pit line or vertical pit line of the cheek of *Osteolepis*. It seems to be continued by the oral mandibular canal which runs forward for some distance, and is the homologue of the mandibular pit line of *Osteolepis*. The dorsal branch from the infra-orbital canal runs backward to the jaw articulation following the general line of the upper border of the palato-quadrato and is continued as the ventral canal on the lower jaw. This is the homologue of the jugal, pre-opercular and mandibular canal of *Osteolepis*. In *Ischnacanthus* there is a canal on the cheek, presumably the jugal; it arises low down from the infra-orbital. Both mandibular canals are present. In *Acanthodes* the oral or quadrato-jugal canal runs parallel to the mouth from the infra-orbital to the pre-opercular canals. The jugal pre-opercular canal arises, not from the infra-orbital canal, but quite dorsally from the main canal. It passes downward, parallel to the hinder border of the palato-quadrato cartilage, to the jaw articulation and is continued as the mandibular canal. This animal, however, has an entirely new canal which arises from the main lateral canal quite posteriorly, passes downward over the operculum not far in front of its hinder margin, and then turns forward, ultimately passing into the mandibular canal. For this, the opercular canal, no homologue can be found.

Most Acanthodians have a ventral lateral line running below the paired fins from the pectoral to the pelvis. I have seen no certain evidence of the occurrence of a dorsal lateral line in any Acanthodian.

Fin Skeleton—The pectoral girdle always has as its chief element a bone associated with the scapular portion of the primary cartilaginous shoulder girdle. This bone in most forms lies so deep that it can be displaced without disturbing the scales which lie above it, but occasionally (*Mesacanthus*, *Diplacanthus*, for example) its outer surface becomes ornamented and it appears to reach out into the skin. The lower portion of the shoulder girdle may be ossified as a coracoid in *Cheiracanthus* and *Acanthodes*, and perhaps other earlier forms.

In *Climatius*, *Euthacanthus*, *Brachyacanthus* and *Parexus* the ventral part of the body in the shoulder region, so far as it is exposed behind the operculum, contains a very variable series of bones. Some of these are certainly of dermal origin whilst all of them may be so. They form in effect a secondary shoulder girdle.

In *Diplacanthus* Mr. GRAHAM SMITH has pointed out to me that the anterior pair of intermediate fin spines has become involved with the ventral part of the shoulder girdle. The chief interest of the animal lies in the way in which the lower end of the scapular and the outer end of the large ventral dermal bone hold the pectoral fin spine firmly, each being attached to a special recessed area on the base of the spine.

The cartilages of the pectoral fin are well shown in *Acanthodes*, with which a less satisfactory specimen of *Ischnacanthus* agrees. In each case the cartilages radiate from a point at the base of the fin. In *Acanthodes* it seems clear that the fin is tribasal. The pro-pterygium supports a single radial and the meso-pterygium another, whilst the small meta-pterygial basal gives attachment to a large bone, the first of a short axis which supports three or four preaxial radials. Immediately distal to the radials lies a double series of dermatrichia which may be ossified in *Ischnacanthus*, *Diplacanthus* and *Acanthodes*. In *Acanthodes* these end whilst still in the basal part of the fin, and another series of dermatrichia which extend out to the fin margin is occasionally preserved.

In all Acanthodians, including rare individuals of *Acanthodes*, it is clear that the fin webs may be coated in part or completely by a regular squamation of extremely small square scales. It thus appears that the dermatrichia may be regarded as ceratotrichia. Nothing is known of the cartilaginous skeleton of the pelvic fin; it possesses ceratotrichia and scales as do the other fins.

The first dorsal fin of *Diplacanthus* is supported by a series of three large basals which support other large bones; these give attachment at any rate for part of their length to a series of short radials. The web of the fin is strengthened by bony ceratotrichia. A similar concentrated basal occurs at the base of the dorsal and of the anal fin in *Acanthodes*.

Thus the general structure of the cartilaginous and dermal skeleton of the fins of Acanthodians resembles that of *modern* sharks in the concentration of the basal elements and the presence of ceratotrichia.

Vertebral Column—In *Diplacanthus* and *Acanthodes* well-developed bony vertebrae were present, but are not well enough preserved to yield detailed information.

SYSTEMATIC POSITION OF ACANTHODIANS

The studies of many embryologists, from BALFOUR to the present day, have shown that the vertebrate head is in the main a segmental structure, each segment possessing pairs of dorsal and ventral nerves, homologous with the dorsal and ventral roots of spinal nerves. This segmentation, which is in origin restricted to the paraxial mesoderm from which the somites are cut off, is designed to allow of lateral flexures of the body and of the mode of swimming based on that possibility. It now seems certain that the earliest vertebrates were microphagous, sieving their food from a water stream, taken in at the mouth and expelled through gill slits, which necessarily passed out from the pharynx between the myotomes arising from the segmental part of the mesoderm. This secondary segmental arrangement of the gill slits thus conforms to the primary segmentation of the somites.

As the gill slits pierce the wall of the gut they necessarily impose a secondary segmentation on the anterior part of the wall of the alimentary canal, which is of lateral-plate mesoderm origin. Thus the primarily unsegmented mesoderm of the head becomes divided into visceral arches, whose segmental arrangement corresponds with that of the somites. Continuation of this reasoning leads to the conclusion that the original mouth lay in front of the segmented part of the body and that it was overhung by a non-segmented prostomial region. Thus a primitive vertebrate should possess a small mouth, followed immediately by a pair of gill slits related to the ophthalmicus profundus nerves. This pair of gill slits is followed by a series similarly supplied by the trigeminus, facialis, glossopharyngeus and the various branchiomic elements of the vagus nerves. STENSIO (1927) has shown that this condition actually existed in the Cephalaspids, and that these animals are directly related to the living lampreys. In these animals the respiratory, or in larvae, the feeding current of water was pumped by the muscles of gill pouches, the pharynx being supported by a skeleton of which the dorsal part was a single continuous bony structure, and the ventral portion though probably stiff was not entirely inflexible. At some stage of vertebrate evolution it became the practice to pump this respiratory stream by volume changes of the pharynx as a whole, brought about by muscles acting on a skeleton which consisted of a series of segmental rods anchored to the vertebral column or basis cranii above and to median elements in the body wall below the gill slits. This visceral arch skeleton and musculature had necessarily a segmentation corresponding to that of the somites of the head, and lay in the gill septa separating the gill slits.

Probably concurrently with these changes, or immediately succeeding them, the vertebrates became macrophagous, eating large prey, presumably still sucking them in and passing the mass backward by muscular movements of the visceral skeleton. The small original mouth proved inadequate and its corners migrated backward, crushing out of existence or absorbing the profundus gill slit, and ultimately settling down at the trigeminal gill slit. The skeleton of the gill septum lying between the

trigeminal and facial gill slits, which consisted of a single chain of four elements, the pharyngo-, epi-, cerato- and hypo-branchial cartilages or bones, thus came to be related to the mouth, and its musculature enabled it to be used as a jaw. In fact it becomes the palato-quadrate and Meckel's cartilage. At this stage the facialis or hyoid gill slit obviously remains of its original size and structure; it was identical in all its important features with the glossopharyngeal gill slit. This is the primitive Gnathostome condition. Continuation of the process of lengthening the mouth, subsequent to the establishment of a definite jaw skeleton, will clearly tend to occlude the middle of the hyoid gill slit by bringing the articular region of the jaws into contact with the main articular region of the hyoid arch. If this contact be established the hinder end of the long jaw will gain a much-needed support through the upper part of the hyoid arch skeleton, a hyomandibular being established and a small relic of the dorsal extremity of the hyoid gill slit surviving as the spiracle. The ventral part of this gill slit would vanish entirely, probably for some reason depending on the time rates of development of the structures involved. This condition is that found in all living fish, Chondrichthyes and Osteichthyes alike, with the possible, though I think highly improbable, exception of the Holocephali.

That the Acanthodians are Gnathostomes is obvious; they all possess well-developed jaws which clearly belong to the trigeminal segment. As in all of them the hyoid gill cleft remains of full size, having indeed a very great dorso-ventral extent, and the hyoid arch cannot in consequence assist at all in supporting the articular region of the jaw, it is evident that they may be very primitive Gnathostomes, ante-dating the development of the spiracle and hyomandibular; the only alternative is to make the gratuitous assumption that the full-sized gill slit has been secondarily reacquired. Which view be correct can be judged from a wider analysis of their structure.

The Acanthodians are the oldest group of Gnathostomes of which there is any record, unless some of the Polish Arthrodeirs be actually of Downtonian age. In any case their acme was in the Lower Old Red Sandstone and they declined from that time onward.

The cartilage ossifications of Acanthodians resemble those of Cephalaspids and Arthrodeirs in being entirely perichondral, the cartilage remaining complete within a thin bony shell. It seems evident that this is the most primitive arrangement known.

The unique neural cranium of *Acanthodes* is very difficult to interpret. The anterior basicranial bone surrounds the hypophysial duct and extends for some distance behind it; this bone must therefore represent the trabeculars. The small bones attached to its anterior end have no obvious homologues in other vertebrates. The posterior basicranial bone must be of parachordal origin. There remains the middle basicranial element. This lies too far back to represent polar cartilages. It is conceivable that it is of acrochordal origin, but the structure seems to lie too ventral to be reasonably interpreted in this way. It is therefore possible that it really represents a detached anterior part of the parachordal, analogous to that found transiently in birds, and may be a relique of an original segmentation of the parachordal cartilage.

The jaws of Acanthodians are remarkable for the fact that in all of them the lower jaw is longer than the upper, exactly as the ventral part of each of the other visceral arches is longer than its dorsal end. The jaws are also remarkable because both upper and lower may be, and presumably at some stage of development always were, formed of two cartilages which, as REIS and JAEKEL have shown, are directly comparable with the corresponding elements of the branchial arches. (The bone supporting the otic process of the palato-quadrato in *Acanthodes* is probably a neomorph.) It is improbable that the palato-quadrato of *Climatius* had any articulation with the neural cranium, but a palato-basal and an otic articulation exist in later forms.

The branchial arches have the primitive gnathostome four-part structure.

The teeth in primitive forms have what is no doubt a primitive Elasmobranch type of replacement and are in some forms extremely scale-like in appearance.

The gill slits open independently to the exterior in early Acanthodians, each gill slit being covered by an operculum arising from its anterior border. Subsequently the mandibular operculum grows back to cover the whole series. Thus the group shows the gradual suppression of a primitive condition.

It has long been recognized that the occurrence of a long series of paired fin spines in the more primitive Acanthodians is a primitive feature. It is explicable on the finfold theory of paired fins, and provides an immediate point of comparison with Anaspids and Cephalaspids, where KIAER has shown that a series of spines or a ridge of enlarged scales can be interpreted in the same way. The fin spines of *Brachyacanthus*, mere bony plates bent round the front margin of the thick fold which constitutes the fin, are directly comparable with the dorsal-fin spine of *Hemicyclaspis* and are clearly very primitive structures. The presence of ceratotrichia and absence of lepidotrichia is another primitive character, not achieved by the Cyclostomes.

It is thus justifiable to regard the Acanthodians as most primitive gnathostomes, belonging to a division of that group characterized by the retention of a full-sized hyoid gill slit. As all other well-known Gnathostomes have this slit reduced to a spiracle, or closed altogether, it is clearly necessary to introduce a new class of vertebrates of a rank equivalent to the Cyclostomata or Pisces for their reception. This new class, which may be termed the Aphetohyoidea (ἄφαιτος, free; ὑο-ειδής, hyoid bone), falls into an intermediate position between the Cyclostomata and the Pisces. It may be assumed to be of ultimate Cyclostome (Ostracoderm) origin, and its possible relationships to true fishes must now be considered.

COMPARISON OF ACANTHODIANS WITH ELASMOBRANCHS

A group of the grade here attributed to the Aphetohyoidea would, on the analogy of the Ostracodermi and the Pisces, be of complex constitution, exhibiting a wide adaptive radiation, and it would necessarily include the ancestors of the true fish, the Pisces. The following discussion is directed to the elucidation of the relationship which the presumed Aphetohyoidean ancestors of the Chondrichthyes bore to the

Acanthodii. The group of Chondrichthyes includes essentially the Elasmobranchii and the Holocephali. I deal with the latter independently and restrict the present section to the true Elasmobranchs.

It is clear that the Upper Devonian fish *Cladoselache*, *Cladodus wildungensis* and *Ctenacanthus clarki* are true Elasmobranchs and that no fish of earlier date, represented by adequate material, can be referred to that class. The Middle Devonian fossils which have been regarded as Elasmobranchs are the spines *Machaeracanthus*, *Gyracanthus* and *Onchus* which are in all probability Acanthodians; *Eczematolepis* which is a Ptyctodont; *Cyrtacanthus* and *Gamphacanthus* which do not at all resemble any known Elasmobranch spines; and a few spines referred to the form genus *Ctenacanthus*, whose relationships are uncertain. In addition there are teeth referred to *Diplodus* which may be the Acanthodian *Doliodus*, and two individual teeth referred perhaps correctly to *Cladodus* but probably incapable of real determination. The Lower Devonian fish remains sometimes regarded as Elasmobranchs are the spines *Onchus*, *Gyracanthus*, *Homacanthus*, *Pinnacanthus*, *Helenacanthus* and *Bulbacanthus*, of which the first three may well be Acanthodians whilst the remainder present no resemblance to any Elasmobranch structure. It thus follows that the earliest known Elasmobranchs are from the Upper Devonian, the group appearing much later than the Osteichthyes.

The early history of the Elasmobranchs though little known was clearly complex; an attempt to discuss it must be founded on the fin structure. *Cladoselache* is a fish apart, characterized by the fact that all the fins, both median and paired, are supported by a single series of parallel radials which extend outward very nearly to their margins and leave unsupported only a very small expanse of fin web, strengthened by ceratotrichia which have not been preserved. All the fins are attached to the body by a very long base, but some concentration of the base of the fin is shown by the occurrence of certain radials which extend in only half-way to the base, and by a fusion of the more posterior basal cartilages of the pectoral fin, each anterior basal supporting its own radial. There are no fin spines. *Ctenacanthus clarki*, a contemporary of *Cladoselache*, is remarkable because it possesses at least one dorsal fin spine. The pectoral fin resembles that of *Cladoselache* in the long extension of its radials all of which extend inward to the base so that there is no evidence of concentration. The fin was interpreted by BASHFORD DEAN as showing a continuous series of basals, one to each radial, but this reading of its structure leaves unexplained the apparent basals attached to the misplaced shoulder girdle, and certain other structures shown in the photograph. Mr MOY-THOMAS has suggested to me that the so-called basals are really the detached basal parts of the radials, and that a still deeper series of largely fused basals may have occurred.

Those Lower Carboniferous Elasmobranchs sufficiently well preserved to be usable are *Sphenacanthus costellatus*, *Tristychius armatus*, *Chondrenchelys problematica* and *Cladodus neilsoni*. It should be remembered that *Pleuracanthus* occurs in the Lower Carboniferous although the well-preserved complete skeletons are Upper Coal Measures, Permian or

Triassic in age. *Cladodus neilsoni* has every appearance of being a direct successor of *Cladoselache*. It is devoid of fin spines, and the pectoral fin differs only by the backward growth of a long metapterygial axis from the group of posterior fused basals which occurs in the latter. The long radials extending nearly to the fin margin are the same in the two fish. *Sphenacanthus costellatus* is a most interesting fish. As SMITH WOODWARD has pointed out, it resembles *Cladoselache* in the extension of parallel radials nearly to the fin margin in all the fins except the first dorsal; but as BROUGH (1935) and MOY-THOMAS (1935 b) have shown, the pectoral fin is tribasal, three elements articulating with the shoulder girdle; the posterior of these seems to me to support a short metapterygial axis. The radials, unlike those of *Cladoselache*, are divided into proximal and distal moieties. I think that BROUGH is correct in holding that this fish is a Hybodont. In later Hybodonts the radials become much shorter, they are indeed restricted to the base of the fin, and those of the pectoral fin are further subdivided. *Tristychius* is of interest because its dibasal pectoral fin still retains radial cartilages, perhaps jointed, which extend out nearly to the margin of the fin and are parallel to one another, agreeing in these respects with *Cladoselache*, *Cladodus* and *Sphenacanthus costellatus*. It seems to me probable that the long metapterygial axis of *Cladodus neilsoni* projected from the body and would thus serve as a morphological ancestor of the "biserial archipterygia" of *Pleuracanthus* and *Chondrenchelys*.

The Coal Measure Elasmobranchs are *Symmorium reniforme* and *Petrodus patelliformis*. Of these the first may readily be interpreted as a Cladodont with an elongated but not subdivided metapterygium. *Petrodus* is more interesting because it is typically tribasal, there being no extended metapterygial axis, and the straight parallel radials are short and jointed, a definite advance on the Lower Carboniferous *Sphenacanthus* and *Tristychius*.

It follows from this analysis that the most primitive known Elasmobranch pectoral fin, from which all others can be derived, is that of *Cladoselache*, and that the most striking of its characters are the parallelism, extension nearly to the fin margin, and unjointed nature of the radials, and the parallel series of short basals.

I have shown that the best known Acanthodian pectoral fin (that of *Acanthodes*) is tribasal and has extremely short jointed radials. In fact it resembles a type of fin, found only in Mesozoic or still later Elasmobranchs, which is the result of a long evolutionary process. The pectoral fin of the Lower Devonian *Ischnacanthus* clearly agrees in its general structure with that of *Acanthodes*. So that even the earlier Acanthodians cannot be compared with the very much more recent primitive Elasmobranchs.

The oldest known Elasmobranch neural cranium is *Cladodus wildungensis*, the only other reasonably well-known Palaeozoic form being *Diacranodus* (*Pleuracanthus*) *texensis*. These two agree very closely indeed with modern Elasmobranchs like *Notidanus* or *Chlamydoselache*. The Elasmobranch neural cranium from the earliest times onward has thus the following qualities: It is composed of cartilage with a characteristic superficial calcification of a single layer of prismatic granules. (No fragment of any Palaeozoic fish of certain Elasmobranch nature shows any other type of calcification, the known

forms are *Cladoselache*, *Ctenacanthus*, *Cladodus*, *Sphenacanthus* and all Hybodonts, *Petrodus*, *Pleuracanthus*, Cochliodonts, Petalodonts and Edestids.) The neural cranium is platybasic, the occipital region being very short and ending abruptly at a nearly transversely placed posterior surface of the otic capsules. The otic capsules are square-cut masses of cartilage confluent with the post-orbital process, forming the transversely placed hinder wall of the orbit. They are deep at their outer surface, and their lateral surface is nearly at right angles to their ventral surface. There is a hyomandibular facet on the outer surface of the otic capsule below the level of the horizontal semicircular canal and often very ventrally placed. The orbits are separated by the relatively wide brain cavity which extends down between them. Each is overhung by well-developed crista supraorbitales and floored by a suborbital shelf which was broad in the primitive forms but becomes reduced when it is cut into by the orbital process of the palato-quadrates. *Pari passu* with this lateral embayment of the suborbital shelf proceeds the appearance and development of the "basal-angle" (Basalecke, GEGENBAUR). The large spheroidal olfactory capsules are separated, often very widely, by the extension forward of the brain cavity as the precerebral cavity, the walls of which support the rostrum and may be reduced to rods of cartilage. The nasal openings are directed ventrally or ventrolaterally, never forward.

Comparison of this account of the fundamental type of Elasmobranch neural cranium with that of *Acanthodes* on pp. 97-102 (with which *Cheiracanthus* is known to agree in essence) will show that the two structures differ as completely as is possible. The Acanthodian neural cranium is bony. The Acanthodian neural cranium is tropibasic. The occipital region of *Acanthodes* is long and passes directly into the otic region. The lateral surface of the otic region bears a horizontal crest, below which it is excavated, passing smoothly into the ventral surface. The post-orbital process is independent of the otic capsule and is connected with the basis cranii by powerful sloping columns of cartilage, perforated by the jugular canal (and in so far agreeing with the post-orbital processes of *Cladodus wildungensis*), which abruptly end the recess on the lateral surface of the otic capsule exactly as do the homologous structures in Palaeoniscids. There are well-developed basipterygoid facets, and a complete absence of the clearly non-homologous articular faces for the intra-orbital processes of the palato-quadrates and the basal angle associated with them. There is an inter-orbital septum, whose slightly expanded lower edge can scarcely be called a subocular shelf. The shape of the snout of all Acanthodians, and the known position of the nostrils in some, show that the olfactory capsules must have been small, in contact with one another, and have had anteriorly directed nostrils.

These differences in the neural cranium reflect a corresponding difference in the brain and in the whole behaviour of the animals. Elasmobranchs are fish in which smell is the dominating sense of distant perception, sight being of minor importance. Acanthodians were clearly creatures in which sight was highly developed and smell of correspondingly less importance.

The Elasmobranch mandibular arch consists of a single palato-quadrate cartilage which anteriorly meets its fellow in the middle line below the neural cranium. It articulates with the suborbital shelf by an orbital process, has no palato-basal articulation, its deepened hinder end does not usually articulate with the post-orbital process by an otic process, and the whole is usually longer than the lower jaw. The lower jaw is a single Meckel's cartilage. (Note: VAN WIJHE has reported the occurrence of two centres of chondrification in the lower jaw of *Squalus*.) The Acanthodian jaw cartilages are superficially very similar to those of Elasmobranchs, but differ in the following ways: The palato-quadrate is composed of two independent bones (presumably representing independent cartilages). The anterior of these is widely separated from its fellow by the ventral edge of the inter-orbital septum; it articulates, if at all, by a basi-cranial facet with the hinder end of the orbito-temporal region, there being no orbital process. There is no otic articulation in the more primitive forms though one appears later. The whole is always markedly shorter than the lower jaw. The lower jaw is ossified in two segments.

In Elasmobranchs the upper element of the hyoid arch is always a hyomandibular. In Acanthodians it is not. In Elasmobranchs the skeleton of each branchial arch is \lessgtr shaped, the pharyngo- and hypo-branchials having their tips directed backward. In Acanthodians it is \gt shaped, the dorsal and ventral elements having exactly the opposite direction. Typical Elasmobranchs have the hyoid cleft reduced to an inhalant spiracle, and the gill slits opening independently to the exterior. In Acanthodians the hyoid cleft is a full-sized gill slit and with those lying behind comes eventually to open into a gill chamber covered by a mandibular operculum.

The above comparisons, which might be greatly extended, are I think sufficient to show that the Acanthodians cannot bear any close relationship to the ancestral Elasmobranchs. It follows that no characters which are common to both Elasmobranchs and Acanthodians can be used as evidence of Elasmobranch affinities of a Palaeozoic and especially of a Devonian fish.

COMPARISON OF ACANTHODIANS WITH ARTHRODEIRS

The group which may be called the Arthrodeirs for my present purpose is composed of the Acanthaspids, Coccoosteomorphi and Ptyctodonts. Our knowledge of the neural cranium of these forms depends entirely on the work of STENSIO (1934). In all known cases the cartilaginous neural cranium remained intact, sheathed for part of its surface by a thin layer of perichondral bone exactly as in Acanthodians. In *Leiosteus* the occipital region is wide and shallow, a condition imposed on it by the general head shape. It is short antero-posteriorly, a condition obviously variable in Arthrodeirs. In *Homosteus* for example it is evident from the specimen figured by HEINTZ (1934, pl. III, fig. 1) that the normal myotomes extended very far forward under the skull roof and that the occipital region must have been very long and slender, transmitting about ten spino-occipital nerves. In *Leiosteus* the notochord was persistent, its

sheath being clasped laterally by the lower ends of the large posterior occipital bone which surrounded the foramen magnum and roofed the hind brain for some distance. This bone is perforated probably for the exit of occipital nerves, its relationship to the vagus is not shown. It may be compared directly with the basi-occipital and ex-occipitals of *Acanthodes* taken together. Apart from proportions the two agree well, indeed very strikingly, in the identical character of the great backwardly opening foramen which is interpreted by STENSIO as the course of the radix aortae. The anterior occipital described by STENSIO (1934) in *Leiosteus* is clearly the homologue of the featureless ventral bone below the otic region of *Acanthodes*.

The only representative of the otic region is a small isolated bone perforated by a jugular canal and a hyoid vein in *Pholidosteus friedeli*. The position of this fragment cannot be fixed and I believe that STENSIO has placed it a good deal too far from the middle line. I base this view on the fact that STENSIO's suggested restoration of the relation of the neural cranium to the dermal roof (STENSIO 1934, text-fig. 15) cannot be right because it makes no allowance for the thickness of the dermal bones.

In *Deinichthys* (HEINTZ 1932, text-fig. 13) the position and possible lateral extension of the neural cranium in front of the occipital region are defined by deep flanges descending from the dermal roof. The similar structures in *Coccosteus* have the same distribution and in addition show entirely different surface features in the regions in contact with the neural cranium and in the rest of the visceral surface of the skull roof. Consideration of this evidence suggests that the "neural process" of HEINTZ in *Deinichthys* abutted against the outer end of a post-orbital ridge which is the most anterior part of the otic region in *Pholidosteus*.

The extent of the dorsal surface of the neural cranium is very clearly defined in *Coccosteus* (cf. HEINTZ 1931, fig. 7) and *Deinichthys*. In the former the position, size and approximately spherical shape of the olfactory capsules are evident from impressions in the dermal roof, and the position of the nostril is shown by the notch in the post-nasal. I have shown that there was a cartilaginous ectethmoid, or preorbital process underlying the latter bones. The diameter and position of the eye can be determined, and the general size, shape and position of the palatal part of the palato-quadrates are fixed by the supra-gnathals which lie attached to its lower surface. The lower surface of the anterior part of the neural cranium can thus be restored with considerable probability; it agrees remarkably with the conditions actually found by STENSIO in *Pholidosteus*. STENSIO's Acanthaspid suggests that a pituitary foramen existed posteriorly. I thus arrive at fig. 23 as a possible neural cranium in *Coccosteus*. In it all the stippled regions represent areas slightly modified from the bones figured by STENSIO. That the brain case so restored presents a very remarkable resemblance to that of *Acanthodes* is obvious and requires no elaborate discussion. It should however be pointed out that the resemblances would remain even if the proportions of the whole structure were changed, and that they are visible in such parts of the structure as are preserved in

STENSIO's materials. It is therefore interesting to look for further evidence to confirm the relationship so suggested.

In *Pholidosteus* the palato-quadrate is surrounded by two perichondral bones, an anterior which articulates with the ventral part of the neural cranium just behind the olfactory capsules, and a posterior quadrate. This condition is directly comparable with that occurring in several Acanthodians. Meckel's cartilage in *Pholidosteus* and

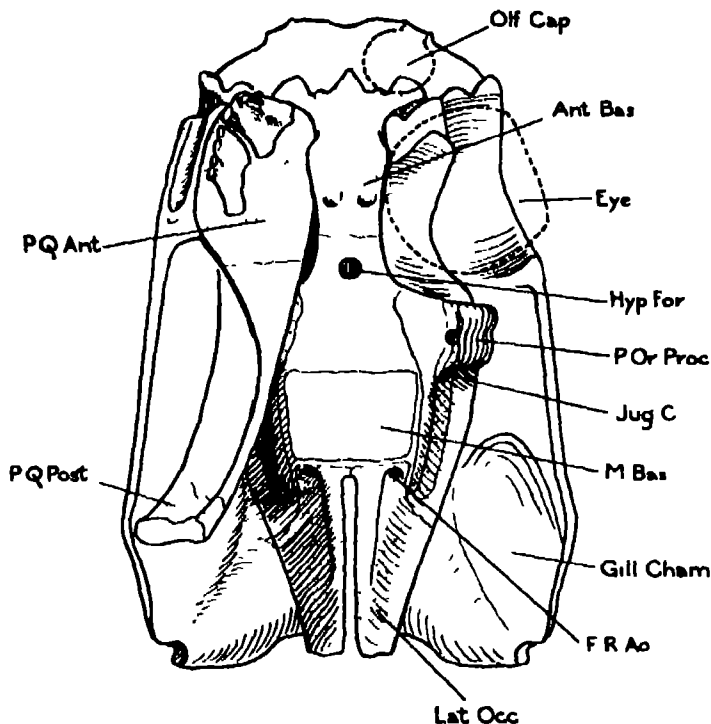


FIG. 23—An attempt to restore the brain case and palato-quadrate of an Arthrodeir by modifying the bones of *Leiosteus* and *Pholidosteus* figured by STENSIO to fit the dermal skull roof and "gnathals" of *Coccosteus decipiens*. $\times 1.0$. *Ant.Bas.* anterior basal; *Eye*, eyeball; *F.R.Ao.* foramen for the radix aortae; *Gill Cham.* the depression on the skull roof which is the roof of the gill chamber; *Hyp.For.* foramen for the hypophysis; *Jug.C.* jugular canal; *Lat.Occ.* lateral occipital; *M.Bas.* median basal; *Olf.Cap.* the olfactory capsule; *P.Or.Proc.* post-orbital bar of the neural cranium; *P.Q.Ant.* and *P.Q.Post.* the two ossifications in the palato-quadrate cartilage.

Leiosteus is also ossified as anterior and posterior bones, again agreeing with many Acanthodians. These conditions do not exist in any Elasmobranch, though a parallel may be found in Palaeoniscids. I have shown that there is evidence in *Coccosteus* that the teeth were arranged in more than one row, a condition which suggests derivation from a dentition comparable to that of early Acanthodians and especially to *Ischnacanthus*.

Gills—It has been shown (WATSON 1934, p. 459) that the Arthrodeirs, especially the Ptyctodonts, were operculate fish with the gill slits opening into an extensive gill

chamber. This was covered externally by an unossified operculum which was attached to the hinder border of the post-suborbital and lower jaw, and carried the lower part of the pre-opercular canal. That this gill cavity extended far up into the head is obvious from the character of the anterior lateral of Ptyctodonts, and there is no real doubt that in *Coccosteus* and *Deinichthys* its upper surface lay immediately below the skull roof on which it leaves a smooth rounded depression. This depression was supposed by STENSIO (1925) to have housed a lateral extension of the otic region of the neural cranium, a view which is obviously at variance with the whole nature of the groove and its inner termination and is not supported by the character of such parts of the neural cranium as are actually known in Coccosteomorphs. HEINTZ, on the other hand, regards it as overlying his "musculus depressor capitis" which I have shown (1934) to have had no existence.

It is clear from examination of actual materials that this depression in *Deinichthys* leads down directly to the gill chamber. Furthermore in *Homosteus* (vide HEINTZ 1934, pl. III, fig. 1) the homologous area was bounded both in front and above by the anterior end of the body musculature, as a gill chamber should be. Thus the gill slits of Arthrodeirs must have had a great vertical extension. Consideration of the extreme antero-posterior compression of the whole gill region in the enormous *Deinichthys* suggests that very long gill slits were necessary to provide an adequate area of respiratory surface. Thus the whole arrangement of the branchial apparatus in Arthrodeirs must have greatly resembled that in Acanthodians.

The most striking feature of the more primitive Arthrodeirs, the Acanthaspids, is the occurrence in them of a long dermal armour over the anterior part of the trunk, and the existence of a long spine projecting laterally from the anterior latero-ventral part of this armour. This spine occupies the position of the anterior margin of a non-existent pectoral fin and is rigidly held by two dermal bones, the antero-lateral and the anterior ventro-lateral, which fit into rabbets on its base. The spine is hollow and variously ornamented with longitudinal ridges, and points or hooks along its inner and outer margins. It is evident from the conditions in Ptyctodonts that the anterior part of the body armour fulfils the functions of a dermal shoulder girdle and is in many ways comparable with the elaborate structures existing in *Climatius* and other primitive Acanthodians. The way in which the large spine of the Middle Old Red Sandstone Ptyctodont *Rhamphodopsis* is held between the antero-lateral and inter-lateral plates is identical, even in the surface detail of the attachments, with that in which the pectoral spine of *Diplacanthus* is held in place. If the spinale of Arthrodeirs be really a pectoral fin spine, and no other explanation of it has ever been suggested, then we should expect to find other fin spines within the group. BROILI has shown (1930a) that in *Lunaspis* there is a spine arising from the anterior median dorsal which is perhaps only a part of that plate and was never related to a fin, and that on the mid dorsal ridge of the body there were two enlarged scales or "stachel-artige Bildungen" which may have been associated with dorsal fins. In *Rhamphodopsis* the median dorsal

clasps the root of a spine which stands up dorsally and is comparable with a fin spine.

Thus it is not impossible that the ancestral Arthrodeirs may have possessed Acanthodian-like fins, with anterior spines. Confirmatory evidence may be afforded by the fact that in *Pterychthiodes*, which STENSIO has shown to be related to the Arthrodeirs, the single dorsal fin has its anterior edge surrounded by a spine which is exactly similar to a fin spine of *Brachyacanthus*. The idea held by TRAQUAIR that this spine was composed of more than one element seems to be erroneous. It is difficult to carry the comparison much further because of the incompleteness of our knowledge, but it may be pointed out that the lateral-line system of the head of *Diplacanthus* is in some ways very reminiscent of that of Arthrodeirs.

In general body form the Arthrodeirs with their macruriform appearance do not recall Acanthodians, the row of ridge scales along the dorsal border of the tail of *Rhamphodopsis*, and the character of the squamation of *Lunaspis* are definite points of difference. It is none the less reasonable to suggest that the two groups possess the same grade of structure and may legitimately be grouped as "Aphetohyoidea". Such reference would not of course preclude a relationship between the Arthrodeirs and the Elasmobranchs or Chimaeroids. The current belief in such an association depends on Dr. STENSIO's comparison of *Macropetalichthys* with the Elasmobranchs, and it is therefore necessary to discuss this fish.

COMPARISON OF ACANTHODIANS WITH MACROPETALICHTHYS

Macropetalichthys is known from STENSIO's classical account (1925) of the structure of the neural cranium, and BROILI's (1933 *b*) excellent description of the whole skeleton. It may be pointed out that the extreme similarity between the neural crania of *Macropetalichthys* and an Acanthaspid seems to establish the existence of a real relationship between the groups to which they belong, despite the very great differences in other parts of the skeleton.

STENSIO compares the *Macropetalichthid* and Elasmobranch neural crania in the following words: "(1) The general shape of the primordial neuro-cranium, especially the tendency to broadening of the ventral surface, partly at the expense of the lateral surfaces. (This is especially the case in the labyrinth region in which the sacculus and perhaps the canalis semicircularis externus had about the same relation to the ventral surface as in *Chlamydoselachus* and most other Selachians.) (2) The position and relations of the olfactory capsule. (3) The presence of a nasal fontanelle on the lower side of the nasal capsule as in *Chlamydoselachus* and certain other Selachians. (4) The presence of the cavum precerebrale. (5) The general shape of the labyrinth, especially with regard to the position of certain of its main parts, as, for instance, the utriculus. (6) The presence of the ductus endolymphaticus and the fact that there probably was a distinct fossa endolymphatica on the dorsal surface of the primordial neuro-cranium beneath the dermal bones. (7) The fact that the ductus endolymphaticus perforated

the dermal cranial roof and had an external opening situated as in certain primitive Selachians (*Chlamydoselachus*). (8) The general shape of the brain as far as this can be restored from the exit of the nerve canals and the shape of the cavum cerebrale. (9) To a certain extent the course and arrangement of the blood vessels and the presence of certain important trunks, as, for instance, the vena hyoidea. (10) The fact that the palato-quadrates as far as can be judged did not articulate with the ethmoid region but must have been suspended below this by ligaments. (11) The probable course of the anterior part of the supraorbital sensory canal. (It should also be mentioned that the sensory canals must have had rather numerous sense organs and that they opened outward with very many tubuli.)"

In 1934 STENSIO, on the basis of a comparison with Arthrodeirs, withdrew his account of the ethmoidal region of *Macropetalichthys* and thereby removed Nos. 2, 3 and 4 from this list. No. 8, the general shape of the brain as determined from the cranial cavity, also falls to be modified. In STENSIO's fig. 10, representing the cranial cavity, the canals for the olfactory tracts are represented as diverging very greatly, in order to reach what were then presumed to be the nasal capsules. This great divergence is not shown in the photographs on pls. XXVII and XXXIII, and cannot have occurred on the new interpretation of 1934, by which the nasal capsules are brought in towards the middle line. As so modified the cranial cavity would very nearly fit an enlarged brain of *Osteolepis*, and cannot be regarded as showing any special Elasmobranch resemblances. Nos. 5, 6 and 7 are concerned with the labyrinth. No. 5, the general character of the labyrinth in *Macropetalichthys*, is as much that of *Acanthodes* as of an Elasmobranch. No. 7, that the ductus endolymphaticus opened on the top of the head is true of *Climatius* as well as of Elasmobranchs. No. 6, that there may have been an expansion of the ductus endolymphaticus between the cartilaginous cranium and the dermal bone of the skull roof, is of very little significance. No. 9 is of very little importance. No. 11, the relation of the anterior end of the supraorbital canal to the nostril, is of course completely changed by the alteration in the position of the nostril, and is in fact as in Acanthodians, and not as in Elasmobranchs. Nos. 1 and 10 are dealt with below. Professor BROILI added to the series of Elasmobranch resemblances of *Macropetalichthys* the following:

- (1) The ventral position of the mouth.
- (2) The structure of the mandible.
- (3) The scales acting as teeth.
- (4) The five branchial arches.
- (5) The thoroughly Selachian-like shoulder girdle.
- (6) The Elasmobranch-like paired fins.

These may be discussed as follows:

(1) *Macropetalichthys* is a flattened, ground living and bottom feeding fish and the ventral position of its mouth may be merely an adaptive character.

(2) The shape and structure of the mandible are related to the feeding habits and may receive the same explanation as (1).

(3) The functional conversion of scales into teeth is merely a primitive feature which is likely to have been practised by the ancestors of all gnathostomes.

(4) The five branchial arches may be paralleled by the five which sometimes occur in *Acanthodes*.

(5) The Selachian-like shoulder girdle is a definite point of resemblance. In some Acanthodians, e.g. *Diplacanthus* and *Cheiracanthus*, there is evidence that in addition to the dorsally directed scapula there was a downwardly and inwardly directed coracoid, which might meet its fellow in a symphysis. In *Climatius* the scapula region is quite like the corresponding structure in *Macropetalichthys*, and a coracoidal extension certainly existed although it is not ossified. The primary shoulder girdle of *Diplacanthus* is in fact like that of *Macropetalichthys*.

(6) The tribasal pectoral fin of *Macropetalichthys* with its short radials is directly comparable, except for the absence of a spine, with that of *Acanthodes* or *Ischnacanthus*. I have already shown that such a fin is not found in the earliest Elasmobranchs and that its occurrence in a Lower Devonian fish is on the whole evidence against Elasmobranch affinities. The very remarkable pelvic fin of *Macropetalichthys* is of course comparable with a primitive Elasmobranch like *Cladoselache*, but it is equally similar to the primitive pelvic fins found in Sturgeons and in *Saurichthys* and *Palaconiscids*.

There remain only two of STENSIO's comparisons.

(10) The non-articulation of the palato-quadrates with the ethmoidal region. Even if true this fact would provide no evidence of Elasmobranch affinities; the palato-quadrates of Acanthodians always end in a free anterior border which lies behind the ethmoidal region.

(1) The general shape of the neuro-cranium with its widened ventral surface was by far the most Elasmobranch-like of all the structures of the fish. This resemblance becomes much less striking when critically considered. The very long occipital region is unparalleled in Elasmobranchs, where indeed it is always exceptionally short. The postero-lateral process arising from the occipital region behind the foramen for the vagus and the distance of that opening from the middle line are unparalleled in Elasmobranchs. The presence of a wide suborbital shelf is very shark like, but is obviously to be associated with the general platybasy and with the small dorsally directed eyes of this bottom-living fish. The very Elasmobranch ethmoidal region has now been shown by STENSIO to have been wrongly interpreted. Thus there are no indubitable Elasmobranch qualities to be found in the very well-known skeleton of *Macropetalichthys*.

It is, however, possible to go further than this purely negative conclusion. The skull roof of BROILI's *M. ? prumiensis* agrees, except that as it is a small young individual the eyes are relatively large, with the specimen from Bundenbach figured by GROSS, and

this latter in turn is not dissimilar to *M. rapheidolabis*. STENSIO has shown that the incompletely known neural cranium of *Epipetalichthys wildungenensis* agrees in all important features with that of *Macropetalichthys rapheidolabis*. Thus there is no difficulty in making the very slight changes in proportion necessary to fit a neural cranium to the skull of BROILI's specimen.

That lateral part of the labyrinth region which is perforated by the jugular canal lies immediately in front of the origin of the pre-opercular lateral line canal and extends forward one-third across the orbit from the posterior margin of that opening.

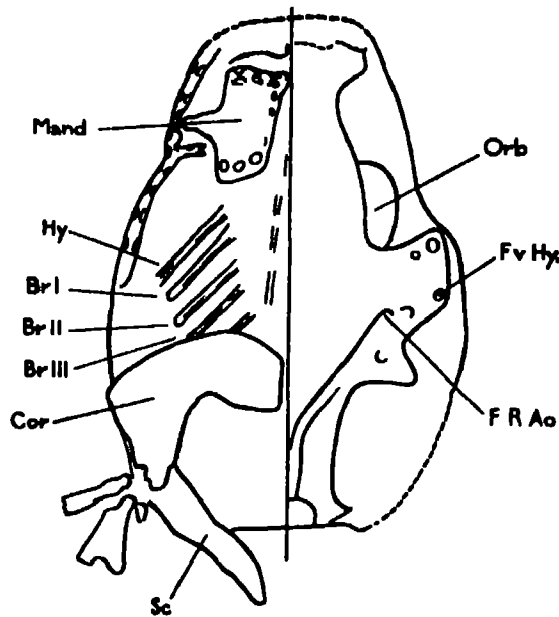


FIG. 24—*Macropetalichthys*. The ventral surface of the head, left side of the figure traced from BROILI (1933a), right side, STENSIO's figure of the neuro-cranium modified in proportions to fit. $\times 1.3$. Br. I–III, the cerato-branchials; Cor. coracoid; Hy. cerato-hyal; F.R.Ao. foramen for the radix aortae; F.v.Hy. foramen for the hyoidean vein; Mand. mandible; Orb. orbit; Sc. scapula.

STENSIO has shown that the upper end of the hyoid arch must have laid below and toward the front of this process, because of the position of the hyoid vein. Trial will show that the posterior end of the most anterior of the branchial arches of BROILI's specimen falls exactly on to this place. It thus seems certain that this is the hyoid arch. It cannot be the first branchial, because if so the upper end of the hyoid arch would be impossibly far forward, especially as the unossified extremity of this arch must also have been directed to some extent forward. We have thus direct evidence that the hyoid arch in *Macropetalichthys* had no suspensory function, but like that of Acanthodians was in function as in homology merely a branchial arch.

In *Macropetalichthys* the pre-opercular lateral line canal passes backward and outward until it is cut off abruptly by the margin of the head shield. In Arthrodeirs it has exactly the same position and was obviously continued on the unossified operculum

which covered the gill chamber. It thus seems probable that in *Macropetalichthys* there was present an operculum which must have been borne on the mandibular arch as is that of Acanthodians. The exact similarity of the hyoid to the branchial arches shows that it cannot have supported the operculum, but must have been over-ridden by it. There is thus direct evidence that the *Macropetalichthyes* are Aphetohyoidea.

COMPARISON OF ACANTHODIANS WITH *GEMÜNDINA*

The systematic position of that very interesting Lower Devonian fish *Gemundina*, and its later relatives *Asterosteus* and *Jagorina*, may now be discussed.

The original account by Traquair was exceedingly tentative and a reconstruction by ABEL founded on it represents a remarkably successful reinterpretation. In 1930 Professor BROILI (1930*b*) published an account founded on much new material and subsequently extended his treatment in a description of two further specimens. He summed up the affinities of *Gemundina* by concluding that it represented a specialized side branch of a subclass of fishes which had a well-developed dermal skeleton, and of which no other representatives were known. The great resemblance to skates and rays is an example of convergence dependent on similar adaptation to a benthonic life. With this view I am in complete agreement, but I believe it possible to go farther with the analysis of the characters of the animal. Professor BROILI's description is so clear and objective, and his illustrations from un-retouched photographs (made from specimens which have not been painted in any way) are so good, that with the experience gained by handling two specimens, the type in Edinburgh and P. 501 in my own collection, I feel justified in offering a reinterpretation of *Gemundina*.

It is clear that all specimens from the Hunsruckschiefer have been to some extent deformed by cleavage, and that in general this deformation is a proportionate reduction in all lengths measured in one direction, in comparison with those in a direction at right angles to it. The ratio of head length to width in the nine specimens which are measurable varies from 0.76 in TRAQUAIR's type to 1.57 in Munich III. The others cover this great gap reasonably completely and suggest that the variation depends almost entirely on the cleavage. The average 1.01 probably represents the normal condition during life.

Münich III (BROILI 1930*b*, pl. II) is in many ways the best preserved specimen, and by making a grid over it and so laterally compressing it in a drawing, I have restored its original shape. It forms the basis of fig. 25A and B.

It seems to me possible to regard the dorsal and ventral parts of the shoulder girdle, and the Flossenträgerplatte of BROILI, as a series of dermal elements quite comparable to the body armour of a Ptyctodont (WATSON 1934, figs. 6 and 7). The most obtrusive structure of the dorsal surface is the remarkable trefoil shaped frame which surrounds the posterior and lateral borders of the skull. The giant specimen (BROILI 1933*a*, pl. I), that figured by BROILI (1930*b*, pl. IV, fig. 1), and the Type, show that the mid-dorsal recess of this frame is bounded behind by a median dorsal bone with a pointed

posterior edge. This is connected to the rest of the armour by long unornamented forward projections of its lateral margins, which lie on the line of the widened hinder end of the neural cranium and its dermal plate. Laterally, forming the side of the mid-dorsal recess in the upper part of the frame, is a wedge-shaped area marked out by two ridges which meet anteriorly at a point immediately behind the dermal bone L^3 of BROILI, just lateral to the tip of the median dorsal. Appearances in the Type specimen make me suspect that this area is an independent anterior-dorso-lateral bone.

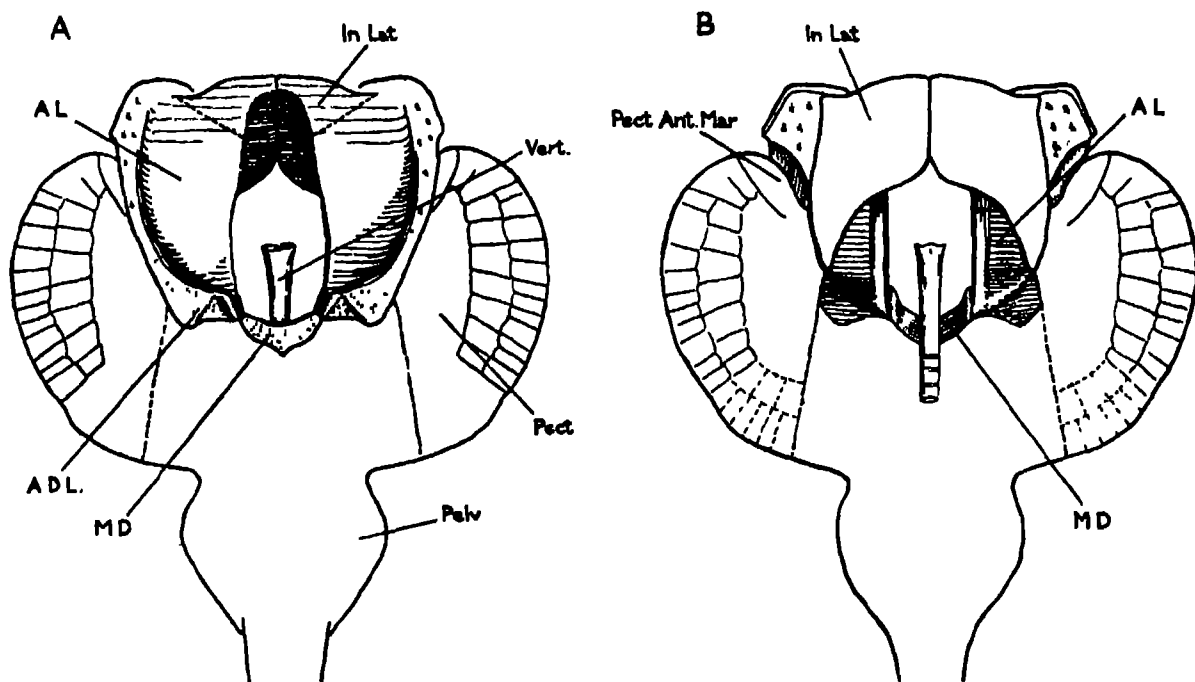


FIG. 25—*Gemundina sturtzi* TRAQ. $\times \frac{3}{4}$. An attempted restoration of the front part of the body with the head removed. A, dorsal; B, ventral surface; A.D.L. anterior-dorso-lateral; A.L. anterior-lateral; In.Lat. inter-lateral; M.D. median dorsal; Pect. pectoral fin; Pect.Ant.Mar. anterior margin of pectoral fin; Pelv. pelvic fin; Vert. vertebral column.

The remaining part of the armour visible from the dorsal aspect has an ornamented external surface of variable width, ending abruptly at the sharp margin of a recessed smooth area, only a narrow strip of which is seen behind the head. This bone may be called the anterior-lateral. As BROILI has recognized, a broad inwardly directed sheet of bone with a thickened admesial edge forms part of the shoulder girdle, it is his ventral part, *c.o.* in the figures. Superposition of tracings of dorsal and ventral views of the same specimen shows that this sheet is part of the same bone (the anterior-lateral) as the ornamented dorsal rim.

The whole arrangement can readily be interpreted on the basis of a comparison with Ptyctodonts, the inwardly directed bone sheet being the hinder wall of a very extensive though flattened gill chamber, the ornamented rim the superficial exposure

of the bone. The exposed part of the anterior lateral widens before it ends, the point of expansion being marked by a distinct notch in the lateral border of the bone, well shown in BROILI (1933*a*, pl. II, left side; 1930*b*, pl. IV, fig. 2); and the Type specimen. The whole bone ends in a thin transversely placed anterior margin which overlaps the outer end of the anterior margin of the Flossenträgerplatte. This region is best shown in BROILI 1930*b*, pl. II, fig. 2, right side; pl. III, fig. 1, left side; and P. 501, D. M. S. Watson collection.

The ventral surface of this dermal armour is almost entirely made by the great Flossenträger, which may be called interlaterals. They meet in the middle line, and probably possessed a dorsally directed back-turned projection which articulated with that sheet of the anterior lateral lying below and behind the gill chamber. The lateral margin is free, but is connected with the lower end of the lateral part of the anterior lateral by an area of skin containing a few large stellate tubercles. This ends abruptly at an embayed edge, best shown in BROILI (1930*b*, pl. III, fig. 1), apparently marking a depression for the anterior corner of the pectoral fin, which is shown (P. 501) by the fact that it can be displaced to have been free. It is evident that a space separated the ventral border of the anterior lateral from the hinder part of the lateral border of the interlateral, and allowed the fin skeleton and musculature to pass from the primary shoulder girdle (of which no trace is preserved) to the fin itself. Comparison of this description, and fig. 25 illustrating it, with the shoulder girdle of a Ptyctodont will show that in principle and indeed in many details the two agree.

It follows automatically from this conclusion that *Gemundina* was an operculate fish, and that the operculum is the large lateral area of the head, containing the plates L^2 and L^3 of BROILI. The structure of the neural cranium, so far as it is known in *Asterosteus* and *Jagorina*, is consistent with this view. It is impossible to determine how far ventrally the slit behind the operculum extended; it seems however most probable that it terminated immediately behind the anterior border of the anterior lateral, the external surface of the operculum and the shoulder there becoming continuous.

Inspection of the published photographs shows that, apart from proportional changes due to crushing and to those which may depend on growth, the extent to which the head (including the lower jaw) is prolonged in front of the anterior lateral is variable. As BROILI has shown the mouth faces dorsally, being in fact most reminiscent of that of an Angler fish *Lophius*. As the centre of the upper jaw is clearly fixed by association with the anterior end of the neural cranium, it is evident that the mouth can only be opened by a protrusion of the lower jaw, and the specimens show that this forward thrust did occur, and that it was associated, as on mechanical grounds it must have been, with a lateral contraction of the mouth. It seems probable that only the "spitz rhombischen Tafelchen" with their stellate tubercles were attached to the lower jaw, and that the "spitzen stachelzähnen" were really carried by the antero-posteriorly widened admesial end of the hyoid.

BROILI has shown that the large admedian part of the hyoid is closely attached to the lower jaw, that its lateral extremity is extended into a slender backwardly and outwardly directed process, and that a series of two or three other slender rods are attached to the hyoid and pass back roughly parallel to this process. As BROILI has stated these can only be the anterior ends of branchial arches. It is possible that the whole unusual arrangement is associated with the protrusible mouth and represents a device for driving the jaw forward.

One very interesting feature of *Gemündina* is the fact that the whole extent of the large pectoral fin is supported by ossified radials, a condition comparable with that of *Cladoselache* and in sharp contrast to that of the Acanthodians. It is probable that the condition in *Gemündina* represents a secondary lengthening of the radials from a stage in which they were short and restricted to the base of the fin.

Professor BROILI's admirable restored figure (1930*b*, fig. 8) represents the animal swimming by causing a series of waves to pass from the anterior to the posterior ends of the pectoral fin. That this conception is correct seems to me altogether probable. But such movements can only be carried out with the necessary power if the intrinsic fin muscles, and the radials to which they are attached, be long. The exactly similar conditions in skates were clearly introduced to subserve the same end. *Gemündina* is also interesting because its single dorsal fin has an anterior spine as in *Pterichthys* or an Acanthodian.

If the foregoing interpretation be true it follows that *Gemündina* is closely related to the Arthrodeira. This conclusion has already been reached by STENSIO. The published figures of *Asterosteus* and *Jagorina* show that their neural crania are far more like those of Elasmobranchs than are the brain cases of *Macropetalichthys* and Arthrodeirs. It will be interesting to consider how far these resemblances depend on the skate-like form of the Rhenanida and how far they are evidence of real affinity between the two groups.

COMPARISON OF ACANTHODIANS WITH CHIMAEROIDS

Very many authors have suggested that the Chimaeroids are the least modified living descendants of the first skull and jaw bearing vertebrates, and that they retain a pre-Elasmobranch condition in the preservation of a complete hyoid arch including an epi- and a pharyngo-hyal. I do not propose to discuss the relationships of this group in any detail, but am compelled to consider the view still held by STENSIO, that they are related to the Arthrodeirs, and the suggestions made by DE BEER and MOY THOMAS (1935) in the following words: "If it could be shown that the jaw suspension of Arthrodira was autodiastyle a good case would have been made out for the existence of an extensive pre-Elasmobranch group from which Acanthodians, Arthrodira, Selachii and Holocephali could be derived. It may be noted that the Holocephali living to-day, with their non-suspensional hyoid arch, are the only survivors of this group to have kept this character, and in spite of their specializations in other directions, they must

be regarded as representatives of the most primitive living gnathostomes." That the Chimaeroids are closely related to the Elasmobranchs is certain (DEAN 1906; GOODRICH 1909) and has never been disputed.

The preceding analysis has shown that there is no evidence of close relationship between any of the known Aphetohyoidea—Acanthodii, Arthrodira, Petalichthids and Rhenanida—and the Elasmobranchs, and that it is hence improbable that the Chimaeroids, even if they were still-living Aphetohyoideans, should be at all closely related to them.

The morphology of one of the most remarkable features of the Chimaeroids—the interorbital septum lying dorsal to the brain cavity—has been most lucidly explained by DE BEER on an embryological basis as the result of a series of changes starting from conditions, found only in Elasmobranchs, of a fenestra precerebralis opening forward into a fossa precerebralis whose walls form the skeleton of the rostrum.

The general build of the otic and occipital regions of the neural skull of Chimaeroids is thoroughly Selachian and differs from that of all other vertebrates. Indeed the only character which has been held to prohibit a derivation of the Holocephali from an Elasmobranch stock is the absence of a hyomandibular and the presence of a pharyngo-hyal. As GOODRICH pointed out (1909, pp. 169–71) there is nothing either in the adult anatomy or in the development to show that the hyomandibular is not fused with the hinder border of the palato-quadrate and with the otic capsule, being indeed the so-called otic process. DE BEER points out that this process lies dorsal to the hyoid vein, instead of being below it as is the head of the hyomandibular in all Selachians. He also identifies a group of ampullae as the spiracular sense organ, and uses them to show that the spiracle lay behind this otic process. I do not think that the identification of this structure is in the least certain or indeed probable, and feel that a migration of the head of the hyomandibular across the vein cannot be excluded.

The branchial arches of Chimaeroids are typically selachian in their backwardly directed pharyngo-branchials, and the hyoid arch agrees entirely with them in its appearance but, according to DE BEER's fig. 1 of a 95 mm. *Callorhynchus*, differs from them in one important respect. Like the opercular cartilage the tip of the "pharyngo-hyal" lies lateral to the efferent hyoidean artery, whilst the true pharyngo-branchials lie mesial to their efferent branchial arteries. The difference between them is of exactly the same character as that which has been shown by DE BEER (1932) to distinguish the pseudo pharyngo-hyal of the Rays from the hyomandibular and the true pharyngo-branchials of these fish. It is thus natural to compare the so-called pharyngo- and epi-hyals of Chimaeroids with the pseudo hyoid arch of Rays, and to regard them as neomorphs fulfilling some functional purpose. That this view is correct is I think shown by the complete disappearance, even in early stages of development, of all traces of the hyoid cleft. Had the Chimaeroids been derived from fish which, like Acanthodians, had a primary complete hyoid arch and a complete pre-hyoidean gill slit, it is very difficult to conceive any reason why the latter structure should have been so completely suppressed.

I think therefore that all the peculiarities of the Chimaeroids can be accounted for by the view that they represent the secondarily free-swimming descendants of a series of primitive, bottom living, *Squatina*-like Elasmobranchs, which in association with durophagous habits produced tooth-plates and an autostylic jaw suspension, and in which eyes were carried up as a kind of turret above their flattened heads. As SMITH WOODWARD has shown, the *Cochliodonts* may be their ancestors.

There remains for consideration the possible relationship between the presumed Aphetohyoidean ancestors of the bony fish and the known members of that group, and the existing evidence showing whether or not the hyostylic and reduced spiracular gill-slit of Elasmobranchs and Osteichthys were independently acquired.

COMPARISON OF ACANTHODIANS WITH BONY FISH

It is evident that the Acanthodians have no close relationship to bony fish. The character of the fins with their concentrated basals and anterior spines is far too advanced to have given origin to those of Palaeoniscids; and it is difficult to relate the dermal skeleton, two members of which clasp the lateral line between them, to that of *Osteichthyes* where the centres of ossification of many important bones lie below neuromast organs. The absence of all dermal ossifications from the palate of Acanthodians is a further important difference.

Nevertheless there is a most curious series of qualities in which the members of the two great groups agree. They are both possessed of replacement and dermal bone. In general build, though not in details of ossification, the neural cranium of Acanthodes is reminiscent of that of Palaeoniscids. The palato-quadrate and Meckel's cartilages are ossified from two independent centres in Acanthodians and in some Palaeoniscids. All the Middle Old Red Sandstone bony fish resemble the Acanthodians in the extremely anterior position of their orbits and the small size of their olfactory organs.

It seems probable that these were characters of the primitive gnathostomes which had been already lost by the ancestors of the Elasmobranchs. If this be the case it seems probable that the *Chondrichthyes* and the *Osteichthyes* were derived from such dissimilar Aphetohyoidean ancestors that the hyostylic and reduced spiracles of each must have been independently acquired and that REGAN is justified in placing the two groups in independent classes or subclasses. If this view be accepted the classification of the lower Craniates will become:

SUMMARY OF CLASSIFICATION

Subphylum.	CRANIATA.
Branch.	AGNATHA.
Order.	Heterostraci.
„	Anaspida.
„	Osteostraci.
„	Cyclostomata.

Branch.	GNATHOSTOMATA.
Grade and Class.	Aphetohyoidea.
Order.	Acanthodii.
„	Arthrodira.
„	Antiarchi.
„	Petalichthyida.
„	Rhenanida.
Grade.	Pisces.
Class.	Chondrichthyes.
„	Osteichthyes.

The description in this paper of the structure of several Acanthodians, and the determination of their systematic position on an adequate though still very incomplete knowledge of the anatomy, will enable them to be used in morphological studies. The most important result is however the verification of a prediction, long implicit in all discussions of the morphology of the lower gnathostomes, that there must have existed a group of vertebrates in which the hyoid gill slit and hyoid arch resembled in their structure the homologous gill slits and arches lying behind them. Such a verification of a prediction which rests on a long chain of reasoning helps to establish the validity of the methods and assumptions of morphology as a mode of thought.

I am very much indebted to the following gentlemen who have allowed me to borrow materials from the collections under their control, and have allowed me to retain such specimens often for years:

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SUMMARY

The Acanthodian fishes are the oldest gnathostomes known and they reached their acme in Lower Devonian times, before any other group.

Their structure has been much less fully described than that of any other group of palaeozoic vertebrates, and the present paper is intended to fill this great gap in our knowledge.

The Acanthodians possess both dermal and perichondral bones, of peculiar character.

They have large eyes and the head has only a very small preorbital portion, with very small olfactory organs and anteriorly directed nostrils. The typical ear has long, slender, semicircular canals and a ductus endolymphaticus open to the exterior.

The neural cranium in general shape recalls that of an Actinopterygian fish, but is differently ossified.

The palato-quadrate has usually a palato-basal articulation, and in advanced forms also an otic attachment. It commonly contains an anterior and a posterior ossification. Meckel's cartilage projects farther forward than the palato-quadrate and also has two centres of ossification.

The hyoid arch is separated from the mandibular arch by a full-sized gill slit, and except for the possible absence of a pharyngo-hyal agrees with the branchial arches in having four bone centres. In primitive forms each arch has a small operculum closing the upper part of the gill slit behind it, but the lower parts of all the gill slits are covered by a mandibular operculum; in advanced forms this comes to override all the gill septa and to rest on the shoulder girdle as does the hyoidean operculum of a teleostome.

An analysis of the whole structure shows that the Acanthodians are the most primitive known gnathostomes, distinguished from all others by the complete development of the pre-hyoidean gill slit. On this basis they are referred to a new grade, the Aphetohyoidea, ranking with the Pisces.

It is shown by a detailed analysis and comparison that the Arthrodira, Antiarchi, Petalichthyida and Rhenanida may be regarded as members of the Aphetohyoidea.

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DESCRIPTION OF PLATES 5-14

All the photographs reproduced in these plates are unretouched enlargements from negatives taken from specimens which, except in the case of some figures of *Acanthodes*, have had no treatment designed to emphasize structures. They are thus evidence to which reference can be made.

PLATE 5

FIG. 1—*Climatius reticulatus* Ag. The head and anterior part of the body, L.O.R.S., Turin Hill. Royal Scottish Museum, Powrie 1891, 92, 198. Ventral surface, the cheek of the left side being exposed. $\times 1.4$.

FIG. 2—*Climatius reticulatus*, the counterpart of the original of fig. 1., B.M.N.H., No. 38596. $\times 1.4$. The dorsal surface of the head, and the cheek, lower jaw and branchial arches of the right side are seen from the inner surface.

Ant.Adm. anterior admedian dermal bone of shoulder girdle; *Ant.Lat.* anterior lateral of shoulder girdle; *Br.Ar.* I, II, etc. the dermal elements of the branchial arches and their opercula; *Circ.Orb.* circum orbital series; *D.Endo Lym.* foramen for the ductus endolymphaticus; *D¹*, dorsal fin spine; *Eye*, the orbit; *Gu.Op.* gular operculum; *Hy.Op.* hyoid operculum; *I¹*, first intermediate spine; *L.L.* lateral-line; *L.Pect.* left pectoral spine; *Mand.* mandible; *Mand.Ray*, a "ray" in the mandibular operculum; *R.L.J.* right lower jaw; *R.Pect.* right pectoral spine; *Sc.* scapula.

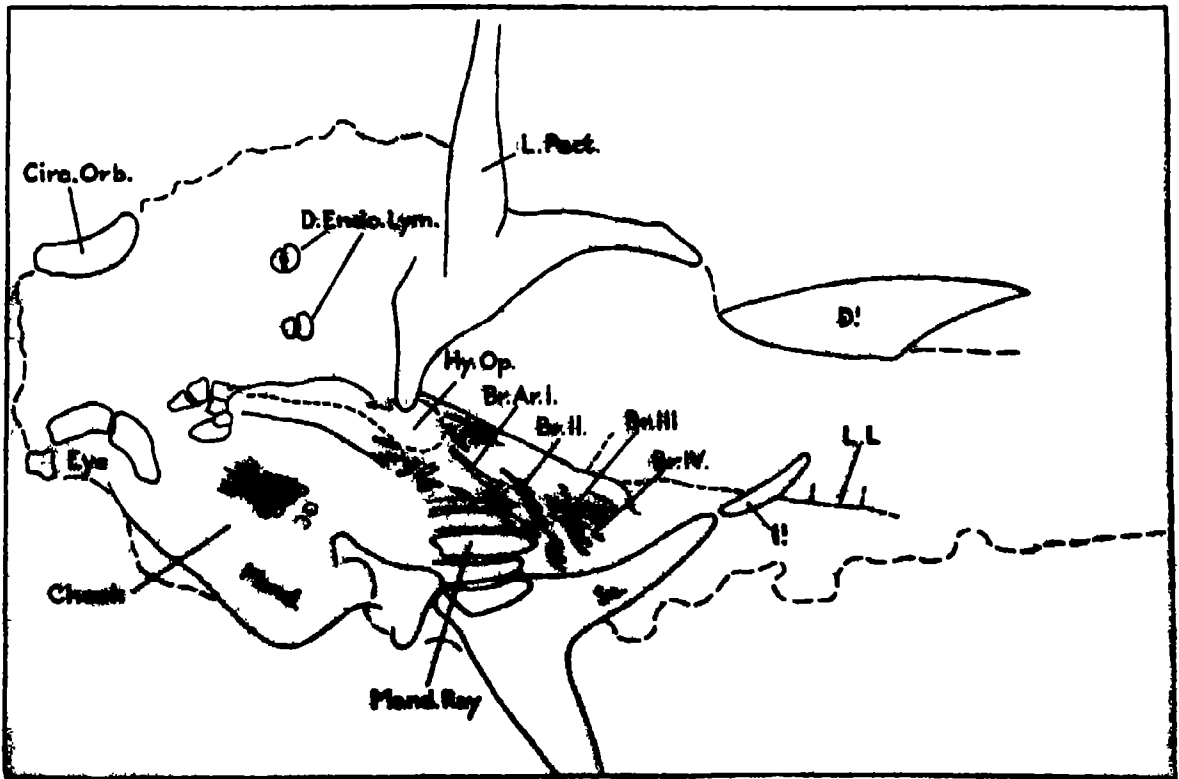
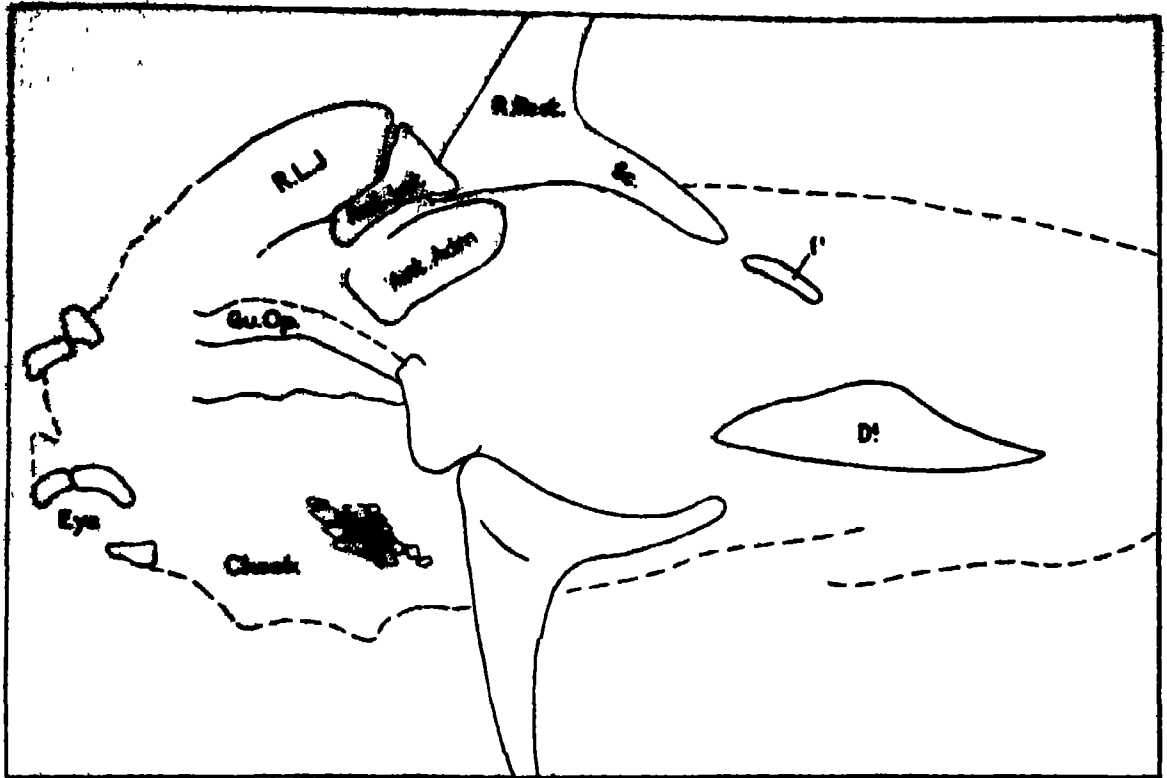


Fig 1

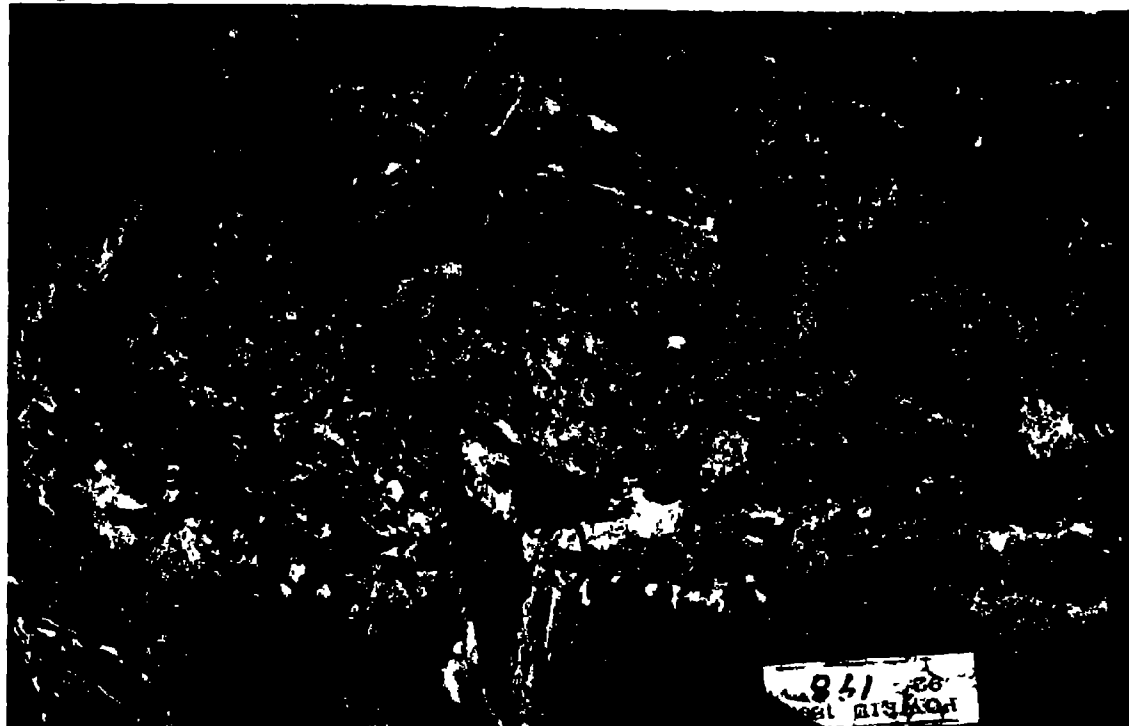


Fig 2



PLATE 6

FIG. 1—*Climatius reticulatus*, L.O.R.S. Turin Hill. Manchester Museum, L. 12096. $\times 0.75$. Complete fish, the head viewed from below, the body in lateral aspect.

FIG. 2—*Climatius reticulatus*, the head of fig. 1, enlarged. $\times 1.72$.

FIG. 3—*Climatius reticulatus*, L.O.R.S. Turin Hill. Royal Scottish Museum, Powrie 1891, 92, 206. $\times 1.24$. The head and shoulder girdle viewed from below. The snout is well shown.

FIG. 4—*Brachyacanthus scutiger* Eg., L.O.R.S. Farnell. Royal Scottish Museum, Powrie 1891, 92, 220. $\times 3.0$. Impression of the left side of the anterior part of the fish, with dark fragments of bone left in the deeper hollows.

Anal, anal fin; *Br.* I, II, III, dermal bones of the branchial arches and their opercula; *Caud.* extremity of the tail; *D*¹, *D*², the dorsal fins; *Eye*, orbit; *Hy.Op.* hyoid operculum; *I*¹, *I*², etc. intermediate fins; *L.L.* lateral-line; *L.Pect.* left pectoral fin; *M.S.* median dermal bone of shoulder girdle; *Mand.Ray*, a "ray" of the mandibular operculum; *Mand. Teeth*, the whorls of teeth on the mandible; *Med.Sc.* median scutes; *P.Q.* palato-quadrates; *Pect.* pectoral spine; *Pelv.* pelvic fin; *R.* ridged bone of shoulder girdle; *R.Pect.* right pectoral fin; *Sc.* scapula.

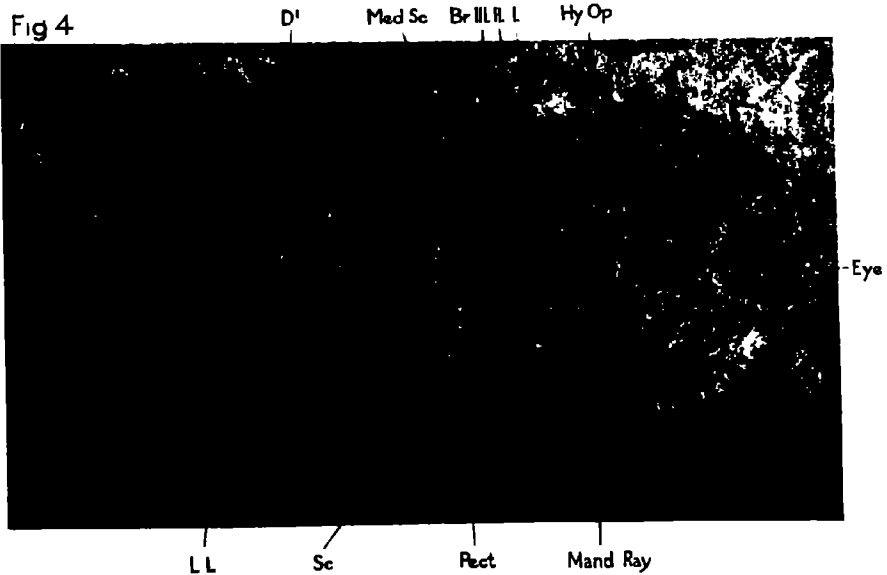
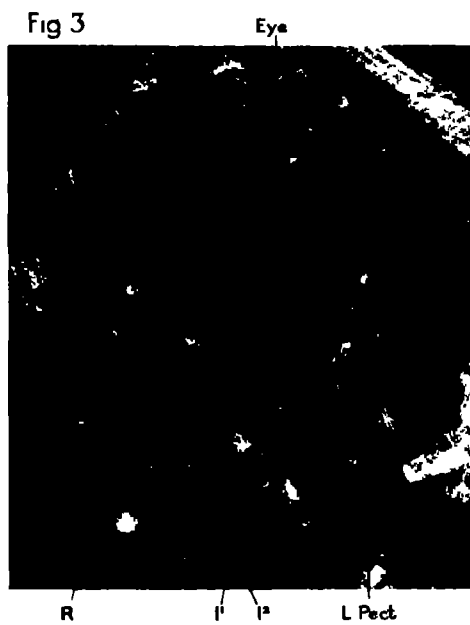
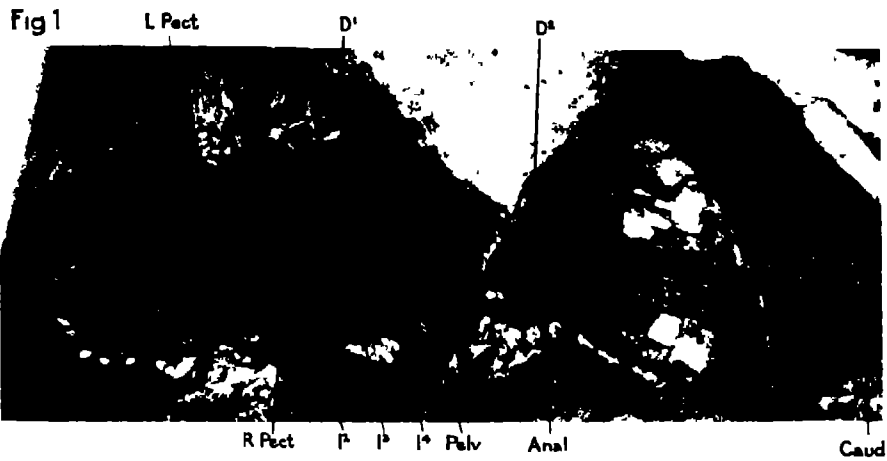


PLATE 7

FIG. 1—*Euthacanthus macnicoli* POWRIE. Type specimen, L.O.R.S. Turin Hill. Royal Scottish Museum, Powrie 1891, 92, 251. $\times 1.0$. The whole fish seen from the left side.

FIG. 2—*Euthacanthus macnicoli*. The head of fig. 1 enlarged. $\times 3.45$.

Anal. anal fin; *Br.Ar.* I, II, III, the dermal bones in the branchial arches and their opercula; *D*¹, *D*², the dorsal fin spines; *Eye*, orbit; *Gu.* gular mandibular rays; *Hy.* hyoid; *I*¹–*I*⁵, the intermediate fin spines; *L.L.* lateral-line; *Mand.C.* mandibular canal; *Mand.Ray*, a "ray" of the mandibular operculum; *Or.C.* oral canal; *P.Op.C.* preopercular canal; *Pect.* pectoral fin; *Pelv.* pelvic fin; *Sc.* scapula.

Fig 1.

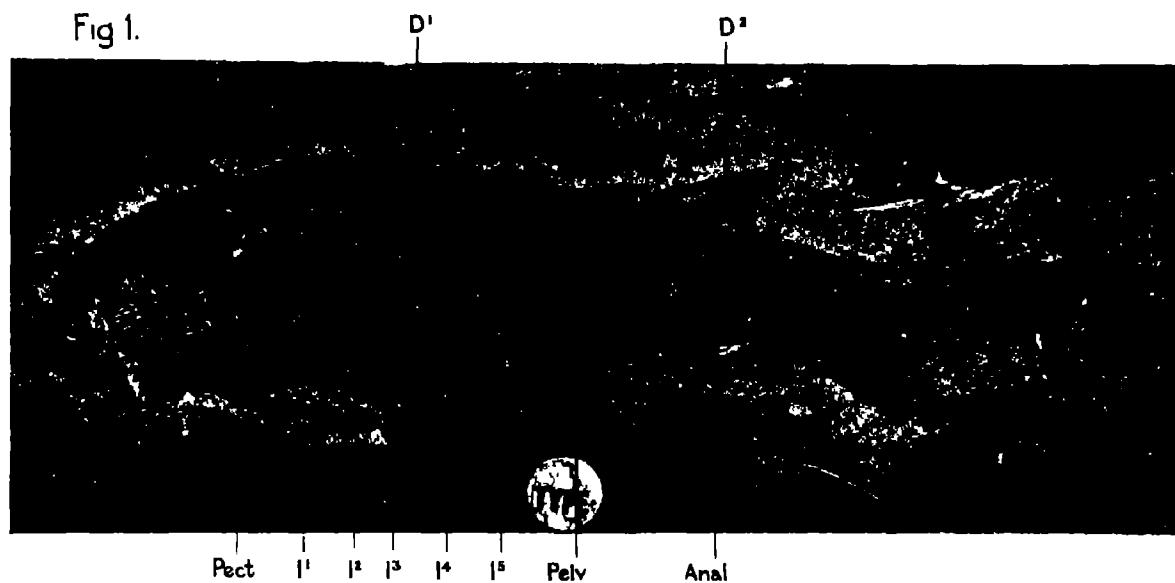


Fig 2

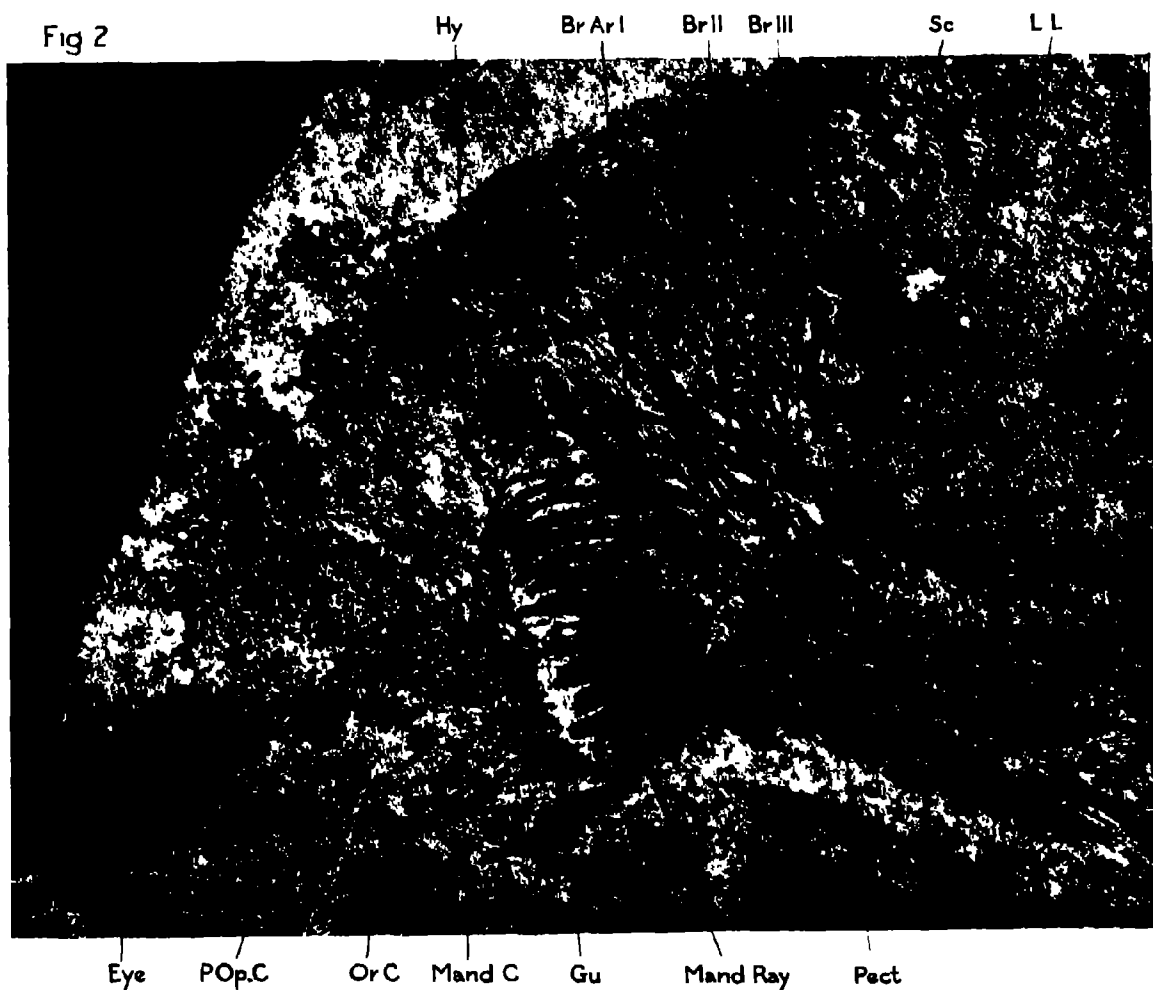


PLATE 8

FIG. 1—*Mesacanthus mitchelli* EG., L.O.R.S. Turin Hill. Royal Scottish Museum, Powrie 1891, 92, 275. $\times 2.5$. A complete young individual from its right side.

FIG. 2—*Mesacanthus mitchelli*, L.O.R.S. Turin Hill. D.M.S.W. Coll., P. 473. $\times 4.7$. Left side of the head of an adult.

FIG. 3—*Mesacanthus mitchelli*, L.O.R.S. Turin Hill. Royal Scottish Museum, 1881, 5, 80. $\times 3.6$. Left side of the head, the palato-quadrates, Meckel's cartilage, and the mandibular operculum well shown.

FIG. 4—*Mesacanthus mitchelli*, the head of fig. 1, further enlarged. $\times 3.8$. Shows especially well the hyoid arch and its operculum.

FIG. 5—*Mesacanthus mitchelli*, L.O.R.S. Turin Hill. Royal Scottish Museum, Powrie 1891, 92, 277. $\times 3.8$. Left side of the head.

Anal, anal fin; *Br.* I, II, III, dermal bones in the branchial arches; *Eye*, orbit; *Gu.* gular mandibular ray; *Hy.* hyoid; *Hy.Op.* hyoid operculum; *I.* intermediate fin; *L.L.* lateral-line; *Mand.Ant.* anterior bone in Meckel's cartilage; *Mand.Post.* posterior bone in Meckel's cartilage; *Mand.Ray*, a "ray" in the mandibular operculum; *Mand.Spl.* mandibular splint; *Otolith*; *P.Q.Post.* posterior bone in the palato-quadrates; *Pect.* pectoral fin; *Pelv.* pelvic fin; *Sc.* scapula; *V.L.L.* ventral lateral-line.

Fig 1.

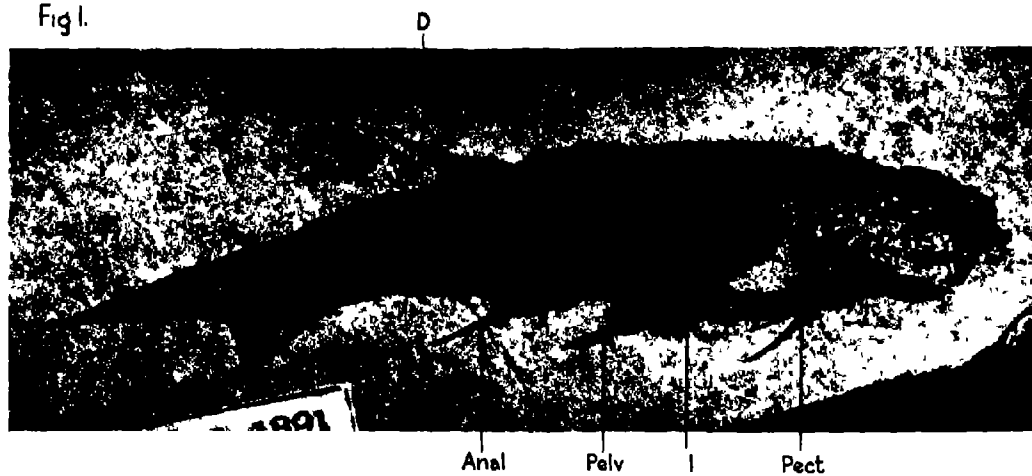


Fig.2.

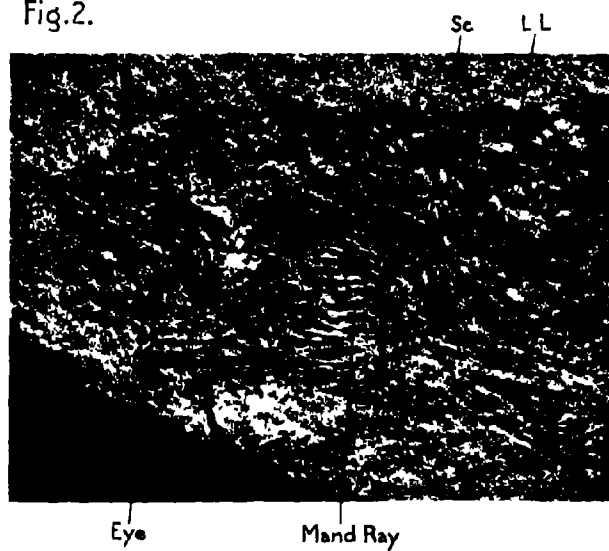


Fig 3.

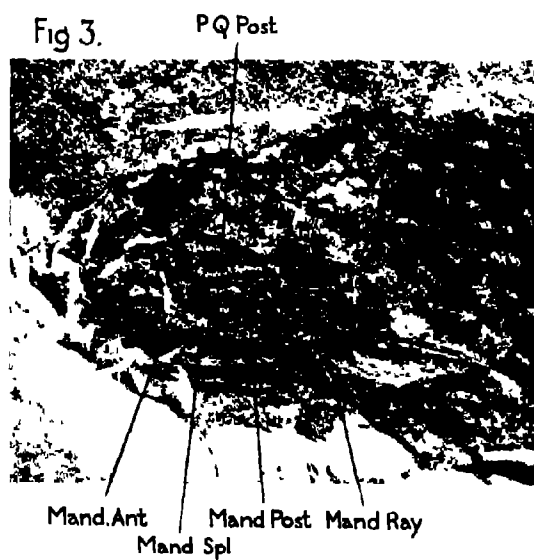


Fig.4

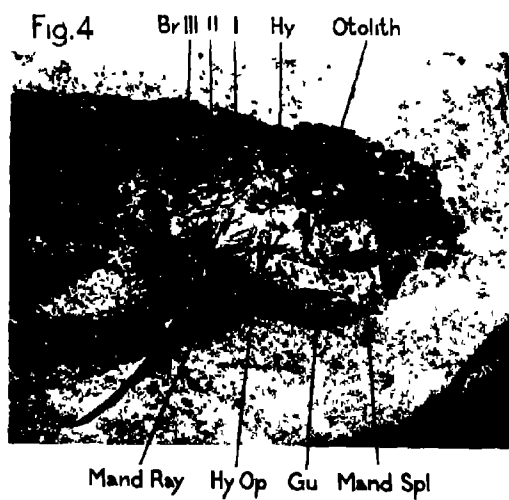


Fig 5

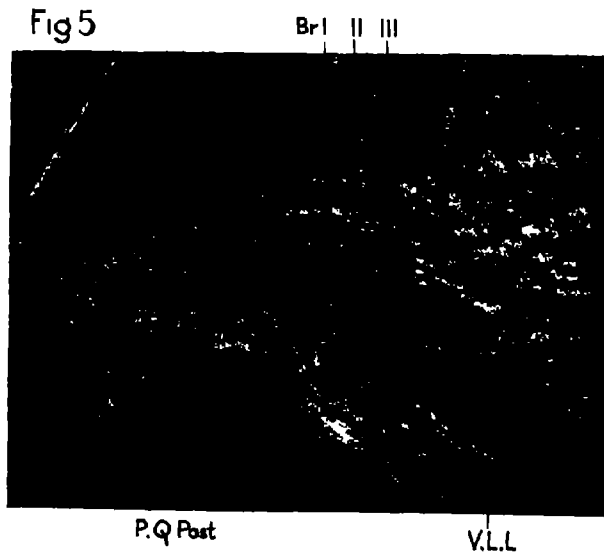


PLATE 9

FIG. 1—*Ischnacanthus gracilis* EG., L.O.R.S. Turin Hill, B.M.N.H. 46305. $\times 2.0$. Left aspect of a small fish lacking the tail.

FIG. 2—*Ischnacanthus gracilis* EG., L.O.R.S. Turin Hill, Royal Scottish Museum, 1887, 35, 2. $\times 2.4$. Left side of the head of a large individual.

FIG. 3a—*Ischnacanthus gracilis* EG., L.O.R.S. Turin Hill, D.M.S.W. Coll., P. 478. $\times 1.4$. Isolated lower jaws seen from the lingual sides, and left posterior palato-quadrato from its palatal surface.

FIG. 3b—The right posterior palato-quadrato bone of the specimen in Fig. 3a, from its outer surface. $\times 1.4$.

FIG. 4—*Ischnacanthus gracilis* EG., L.O.R.S. Turin Hill, D.M.S.W. Coll., P. 481. $\times 2.6$. Right side of the head of a small fish.

FIG. 5—*Ischnacanthus gracilis* EG., L.O.R.S. Turin Hill, D.M.S.W. Coll., P. 297. $\times 1.15$. The tail of a medium-sized individual.

Anal, anal fin; *Ant.Mand.D.* fused whorl of symphysis mandibular teeth; *D¹*, *D²*, the dorsal fin spines; *Eth.C.* ethmoidal commissure; *Eys*, orbit; *L.L.* lateral-line; *L.P.Q.* left posterior palato-quadrato; *Mand.Ant.* anterior mandibular ossification; *Mand.C.* mandibular canal; *Mand.Op.* mandibular operculum; *Mand.Post.* posterior bone in the mandible; *Oral.C.* oral canal; *Pect.* pectoral fin spine; *Pelv.* pelvic fin spine; *Sc.* scapula.

Fig 1.

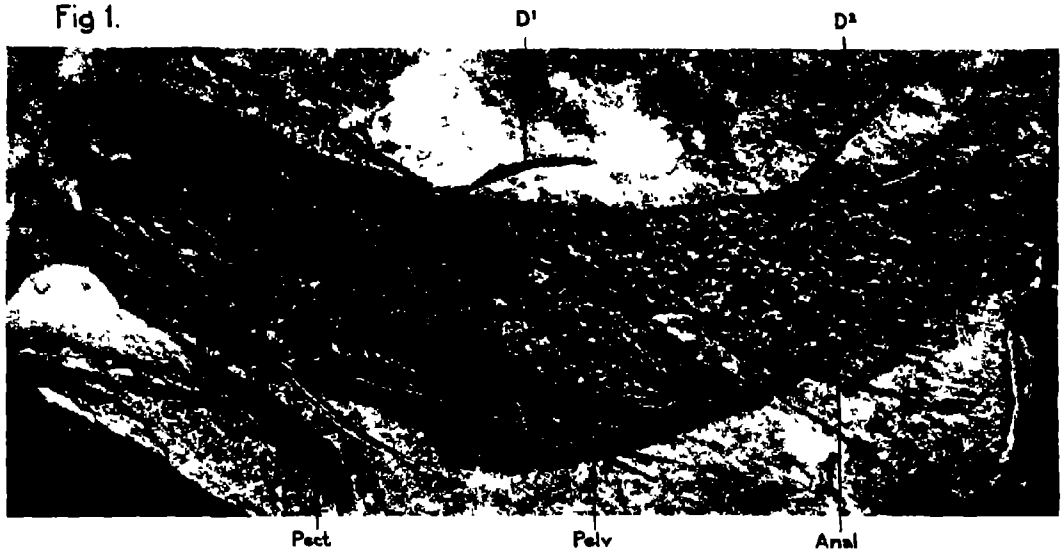


Fig 2.

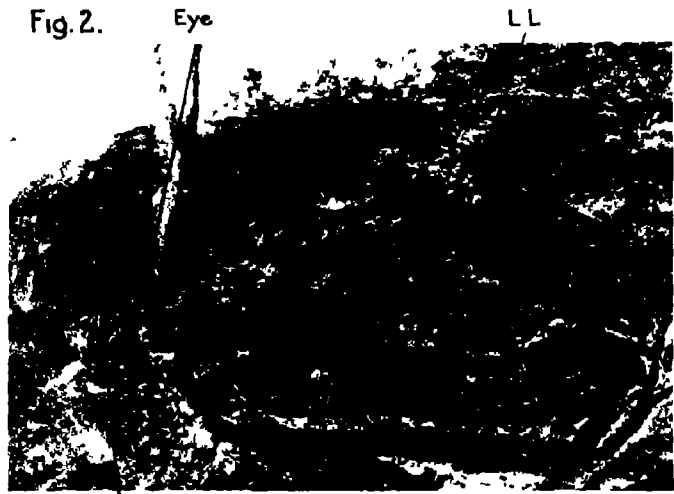


Fig 3a

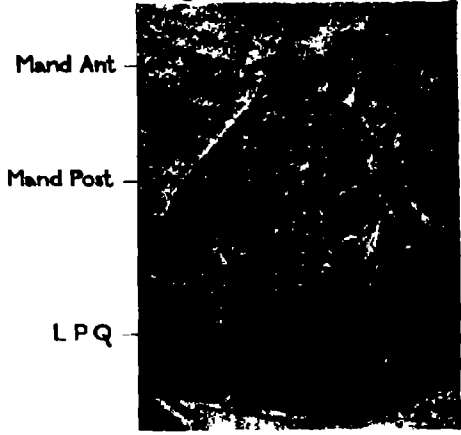


Fig 3b



Fig 4

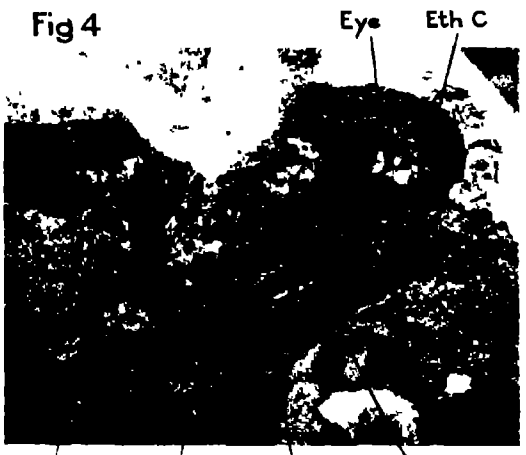


Fig.5.

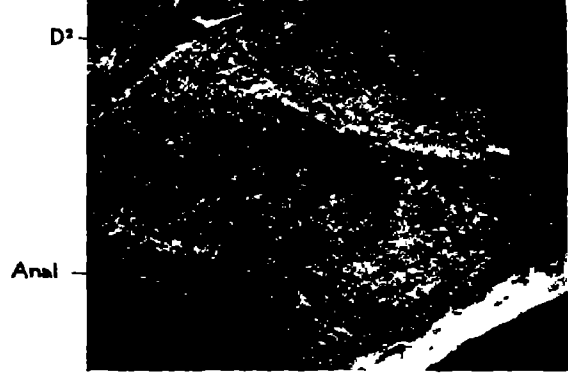


PLATE 10

FIG. 1—*Diplacanthus striatus* AG., M.O.R.S. Tynet Burn, Royal Scottish Museum, Powrie 1891, 92, 334. $\times 2.2$. Left aspect of a fish unique in possessing extensive ossifications in the visceral arches, vertebral column, and basals and radials of the first dorsal fin, and in showing calcified ceratotrichia in the first dorsal and pectoral fins.

FIG. 2—*Diplacanthus striatus* AG., M.O.R.S. Tynet Burn, D.M.S.W. Coll., P. 299. $\times 2.3$. Complete fish showing the check, intergular space, pectoral girdle, fin spines and tail.

Adm.Pect.Sp. admedian pectoral spine; *Anal*, anal fin spine; *Cor.* coracoid; *D¹*, *D²*, the dorsal fin spines; *Eye*, orbit; *I.* intermediate fin spine; *L.L.* lateral-line; *Lat.Pect.Sp.* lateral pectoral spine; *Mand.* ossified Meckel's cartilage; *Mand.Spl.* mandibular splint; *Pelv.* pelvic fin; *Sc.* scapula.

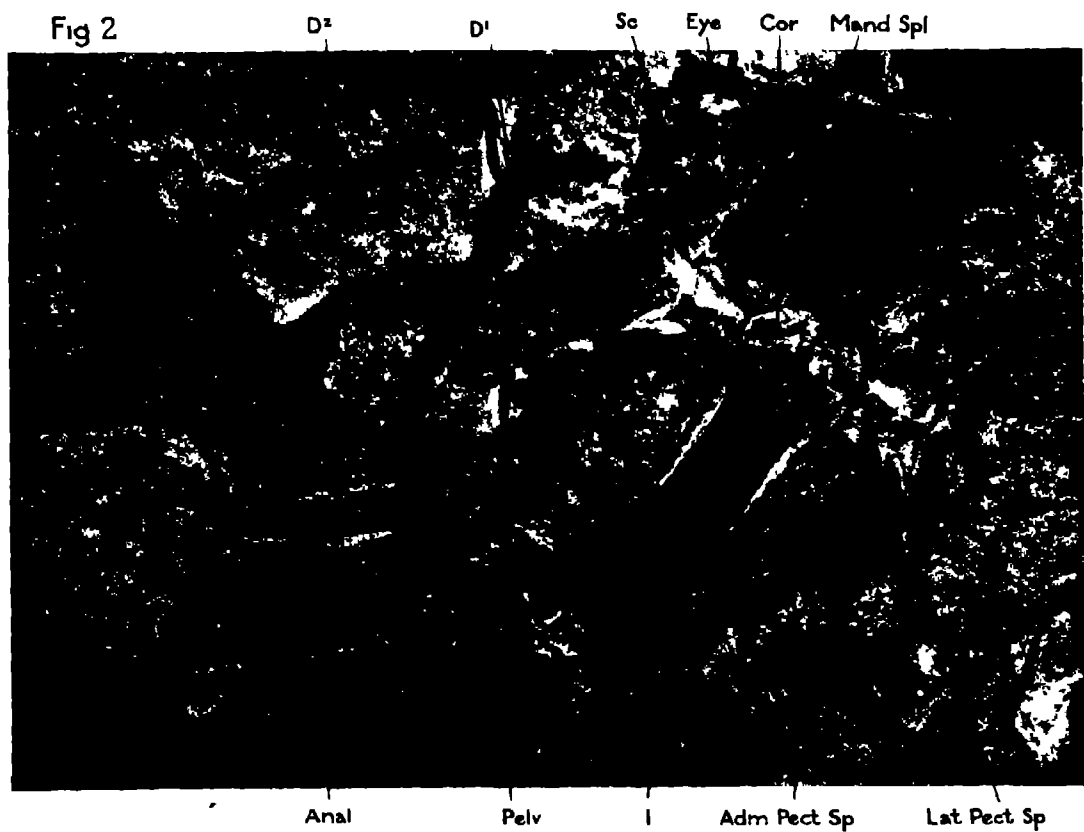
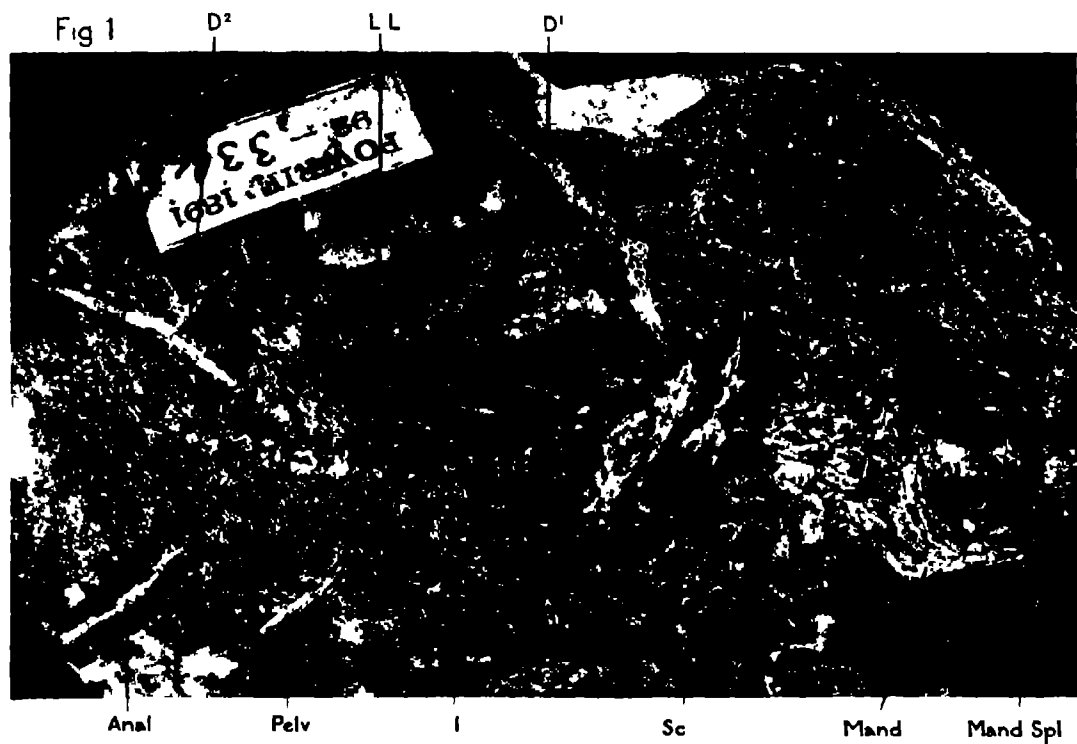


PLATE 11

FIG. 1—*Diplacanthus striatus*, M.O.R.S. Tynet Burn, D.M.S.W. Coll., P. 300. $\times 3.0$. Anterior part of a fish in which the ventral surface from the lower jaws to the pectoral fins is seen from the outer surface, and the lateral and dorsal surfaces of the head are spread out on the left of the figure, represented mainly by impressions of their inner surfaces. Part of the infilling of the lateral line canals remains.

FIG. 2—*Parexus incurvus* AG., L.O.R.S. Turin Hill, Manchester Museum, L. 12097*b*. $\times 1.5$. A nearly complete fish from the left side.

Adm.Pect.Sp. admedian pectoral spine; *Anal*, anal fin spine; *Cor.* coracoid; *D¹, D²*, dorsal fins; *Derm.Pl.* dermal plate of the shoulder girdle; *I¹, I², I³*, intermediate fin spines; *Inf.Or.C.* infra-orbital canal; *Inf.Or.C.b.* posterior median branch of the infra-orbital canal (STENSIO); *Lat.Pect.Sp.* lateral pectoral fin spine; *Mand.Spl.* mandibular splint; *Oc.C.* occipital cross commissure; "*Op.*" large opercular plate; *P.O.* post-orbital plate; *Pect.* pectoral fin spine; *Pelv.* pelvic fin spine; *Sc.* scapula; *Sup.Or.C.* supra-orbital canal.

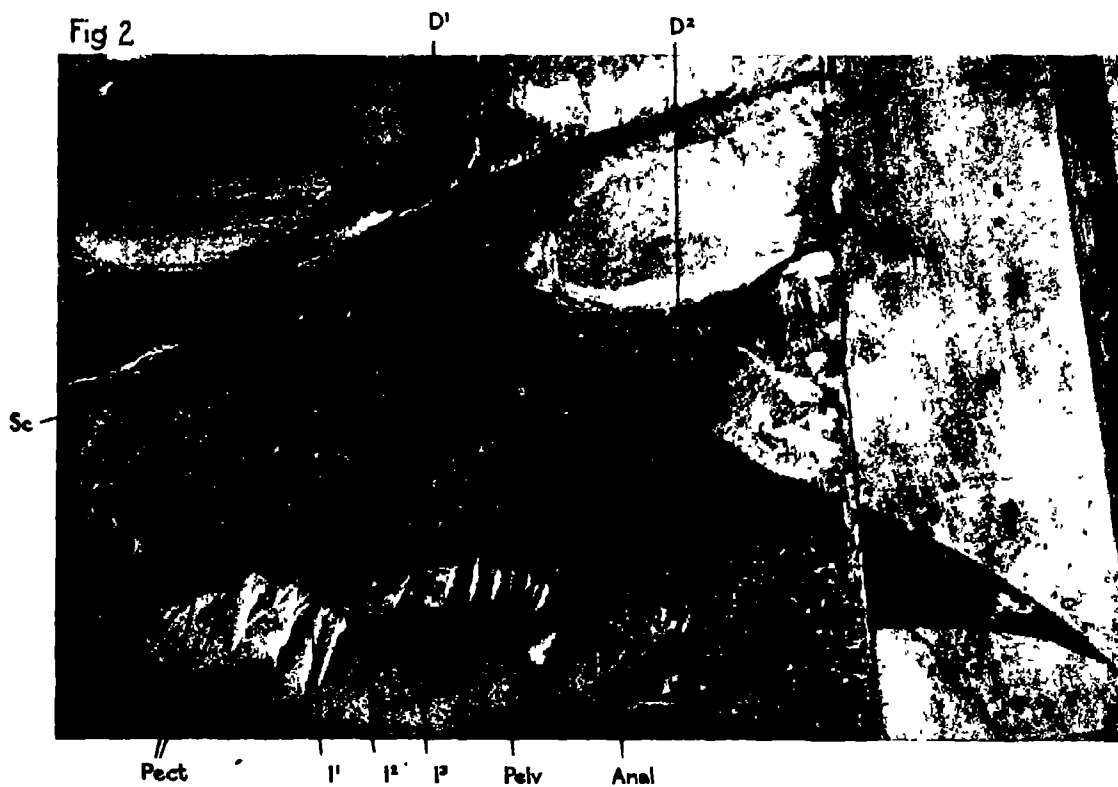
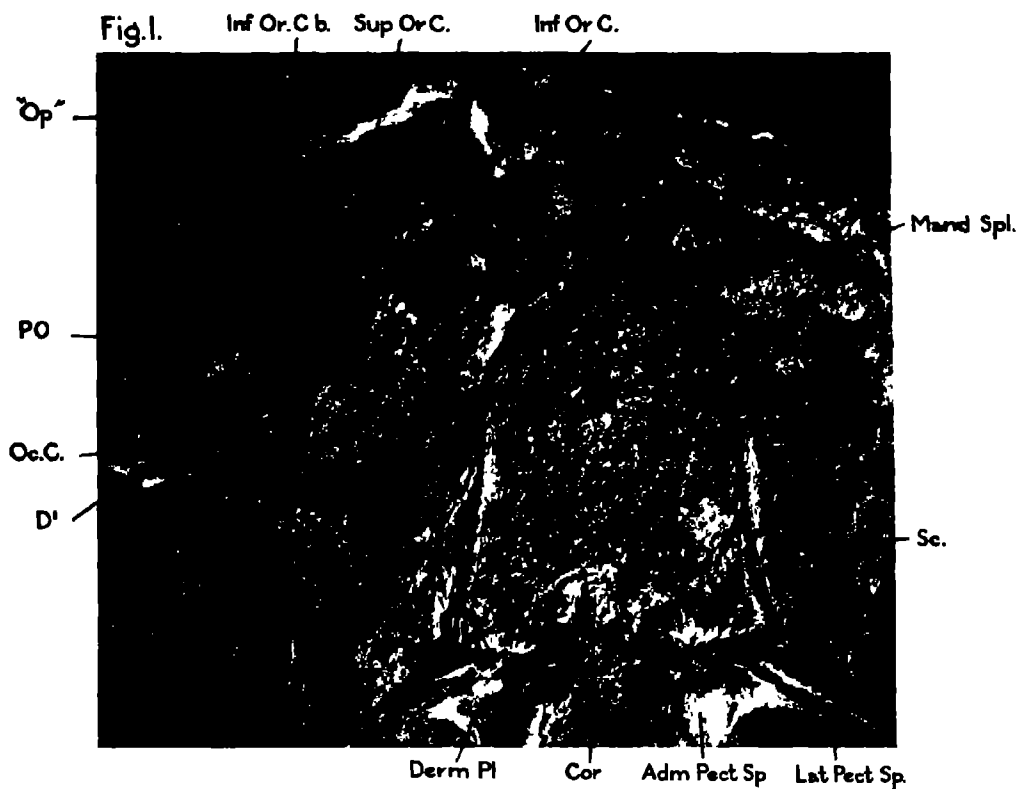


PLATE 12

FIG. 1—*Cheiracanthus murchisoni* Ag., M.O.R.S. Gamrie, D.M.S.W. Coll., P. 492. $\times 3.0$. Right side of the head showing the mandibular operculum, and lower jaw.

FIG. 2—*Cheiracanthus murchisoni* Ag., M.O.R.S. Gamrie, U.C.L. Zoo. Dept., C. 21. $\times 2.0$. Isolated right palato-quadrate from its outer surface.

FIG. 3—*Cheiracanthus murchisoni* Ag., M.O.R.S. Tynet Burn, B.M.N.H. 43273a. $\times 2.5$. Posterior part of the head to show the mandibular operculum dragged down so as to expose the dorsal end of the hyoid and the slender ossifications in the branchial arches.

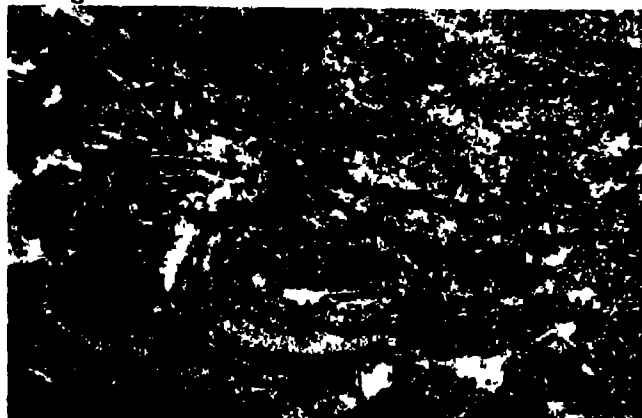
FIG. 4—*Cheiracanthus latus* Eg., M.O.R.S. Tynet Burn, B.M.N.H., P. 3253. $\times 1.9$. The head, mainly an impression of the right surface.

FIG. 5—*Cheiracanthus latus* Eg., M.O.R.S. Tynet Burn, Imperial College, Geol. Dept. $\times 2.2$. The head, mainly an impression of the right surface, showing the row of small bones which forms the border of the mouth, and the jaw elements.

FIG. 6—*Cheiracanthus latus* Eg., M.O.R.S. Tynet Burn, D.M.S.W. Coll., P. 509. $\times 2.4$. The right shoulder girdle and pectoral fin spine from without.

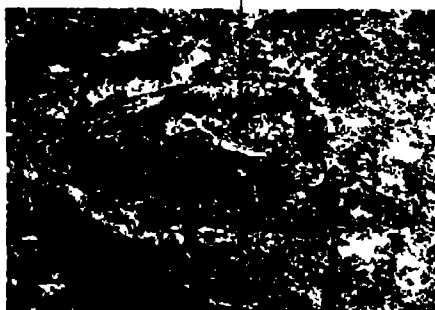
Br. I, II, III, dermal bones of the branchial arches; *Circ.Orb.* circumorbital bones; *Cor.* coracoid; *Gu.* gular rays; *Hy.* upper end of the hyoid arch; *L.L.* lateral-line; *Mand.* the ossification in Meckel's cartilage; *Mand.Ray*, a "ray" of the mandibular operculum; *P.B.Art.* palato-basal articulation of the palato-quadrate; *P.Q.* palato-quadrate; *P.Q.Vac.* vacuity in the palato-quadrate; *Pect.* pectoral fin spine; *Sc.* scapula.

Fig 1



Mand Ray Mand Circ Orb

Fig 2



PQ Vac

PB Art

Fig 4



LL

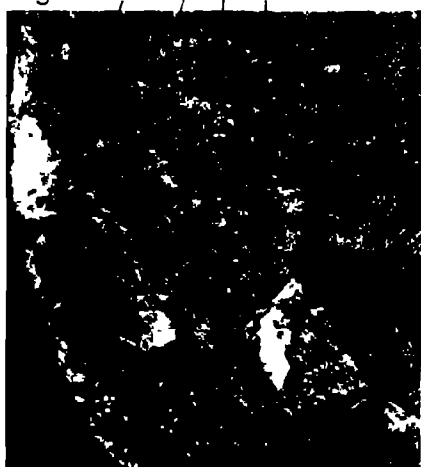
Sc

Gu

Mand

Mand Ray

Fig 3



Hy Bri II III

LL

Sc

Mand Ray

Fig 5



PQ

Mand

Mand Ray

Fig 6



Sc

Pect

Cor

PLATE 13

FIG. 1—*Acanthodes Wardi*. M.C.M. Knowles Ironstone, Longton, Staffordshire, Manch. Mus. LL. 181. $\times 1.4$. Fragment of a head showing the series of gill-rakers on the ventral part of the hyoid arch.

FIG. 2—*Acanthodes* sp. Lebach shales. D.M.S.W. Coll., P. 323. $\times 1.13$. Gelatine cast of the head from the left side, showing the left palato-quadrato and lower jaw and other structures.

FIG. 3—*Acanthodes* sp. Lebach shales. D.M.S.W. Coll., P. 495. $\times 1.25$. Gelatine cast of the brain case viewed from below. Cf. text-figs. 17 and 18.

FIG. 4—*Acanthodes* sp. $\times 1.15$. The specimen shown in fig. 3, from the dorsal aspect, a gelatine cast from the counterpart.

FIG. 5—*Acanthodes* sp. Lebach ironstones. D.M.S.W. Coll., P. 498. $\times 1.42$. To show the pectoral fins.

FIG. 6—*Acanthodes* sp. Lebach ironstones. Berlin Museum, unnumbered. $\times 1.4$. The tail of a small individual.

Ant.Bas. anterior basal; *Ant.Os.* anterior bones of the neural cranium; *Dor.Os.* dorsal bone of the neural cranium; *Ep.Hy.* epi-hyoid; *Hy.Gill-raker*, gill-raker on the hyoid arch; *Hyp.For.* hypophysial foramen; *Lat.Oc.* lateral occipital; *Mand.Ant.* anterior bone in Meckel's cartilage; *Mand.Post* posterior bone in Meckel's cartilage; *Mand.Ray*, a "ray" in the mandibular operculum; *Mand.Spl.* mandibular splint; *M.Bas.* middle basal bone in the neural cranium; *Mes.Pt.* mesopterygium; *Met.Pt.* metapterygium; *Oc.N.* foramina for occipital nerves; *Ot.Pr.Art.* articulation of the otic process; *Pal.Bas.Art.* palato-basal articulation; *Pal.Q.Post.* posterior bone in the palato-quadrato; *Pal.Q.Ot.* otic process of the palato-quadrato; *Ph.Br. I*, first pharyngo-branchial; *Post.Bas.* posterior basal bone in the neural cranium; *Pro.Pt.* propterygium; *Rad.* radial; *X*, notch for the tenth nerve.

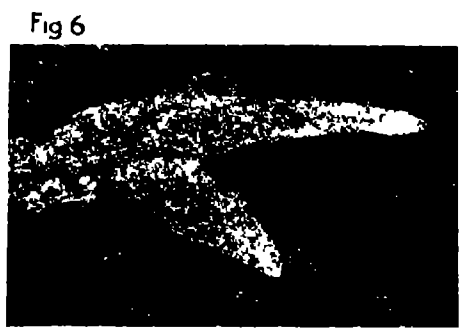
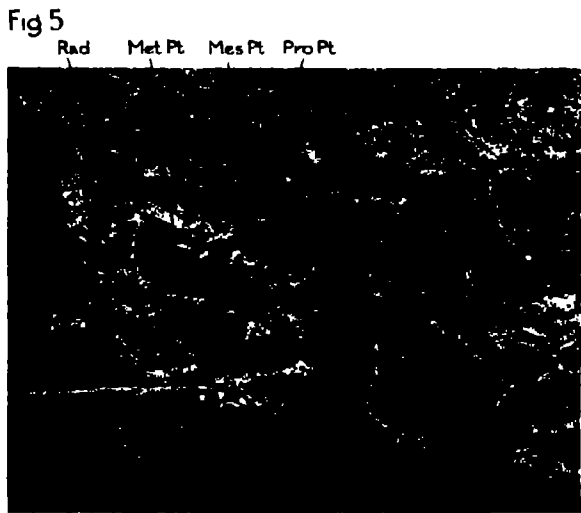
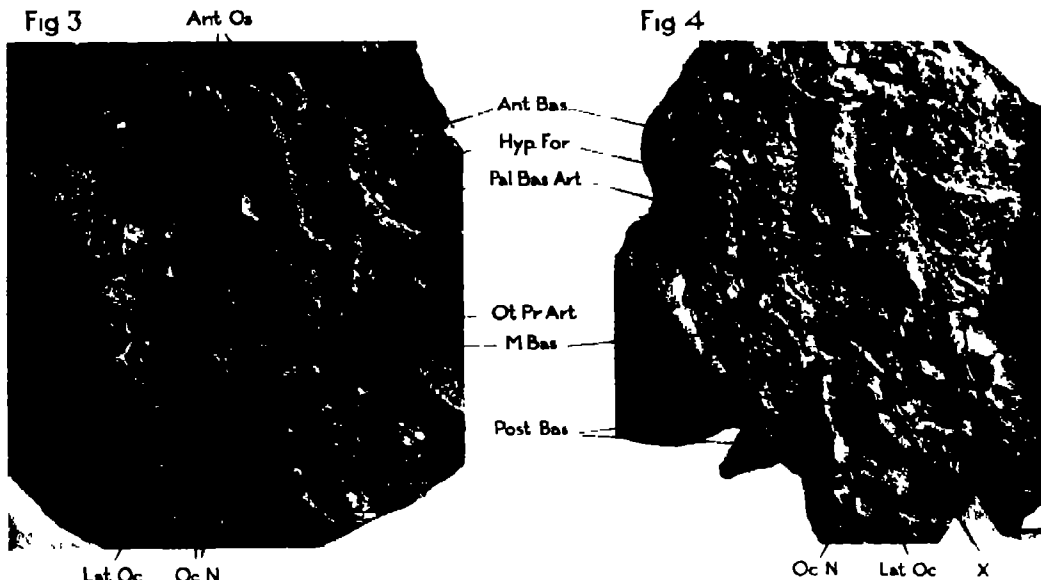
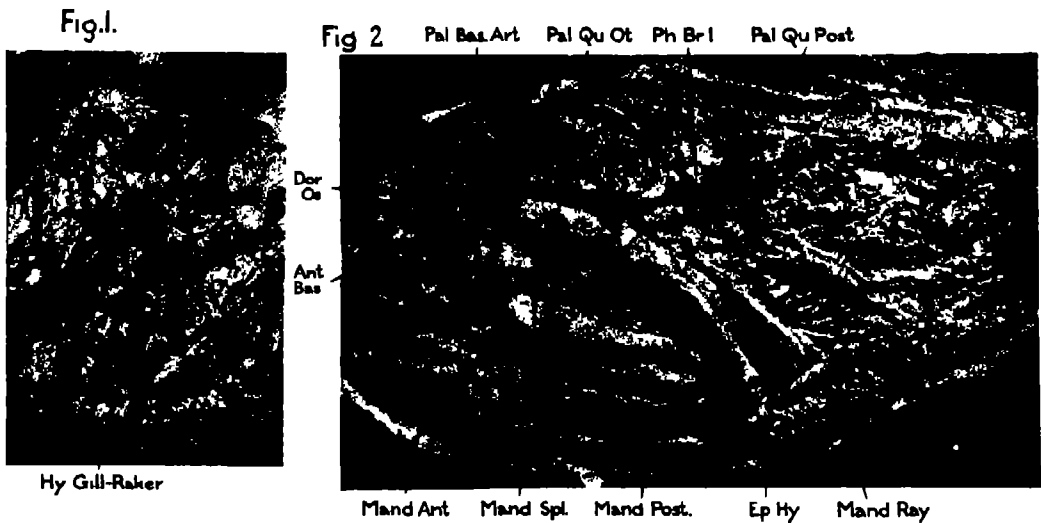


PLATE 14

Photographs of medium sized specimens of *Acanthodes* from the Lebach ironstones of the Saar, Lower Permian. It is probable that each specimen is specifically different from the others.

In every figure except 6 some part of the lateral-line system retains in cavities traces of white paint.

FIG. 1—D.M.S.W. Coll., P. 496. $\times 1.15$. Head viewed from the right. Compare text-fig. 20D.

FIG. 2—Royal Scottish Museum 1891, 42, 3. $\times 1.52$. Head viewed from the left.

FIG. 3—D.M.S.W. Coll., P. 498. $\times 1.2$. Head viewed from the right. Compare text-fig. 20C.

FIG. 4—B.M.N.H., No. 22658a. $\times 1.3$. Compare text-fig. 20B.

FIG. 5—D.M.S.W. Coll., P. 494. $\times 1.05$. Head viewed from the left. Compare text-fig. 20A.

FIG. 6—Royal Scottish Museum, unnumbered. $\times 1.08$. Head from the left.

Br. I–IV, the branchial arches, usually only represented by the gill-rakers; *Cor.tr.* ceratotrachia; *Cor.* coracoid; *Ear*, the semicircular canals; *Ep.Hy.* epi-hyal; *Eys*; *Gu.* gular rays of the mandibular operculum; *Hy.Gill R.* hyoid gill-raker; *Inf.Or.C.* infra-orbital canal; *L.L.* lateral-line; *L.Sup.Or.C.* left supra-orbital canal; *Mand Ant.* anterior bone in Meckel's cartilage; *Mand. Post.* posterior bone in Meckel's cartilage; *Mand.Ray*, a "ray" in the mandibular operculum; *Mand.Spl.* mandibular splint; *Op.C.* opercular canal; *P.Op.C.* pre-opercular canal; *P.Q.Ant.* anterior bone in the palato-quadrato; *P.Q.Ot.* otic process of the palato-quadrato; *P.Q.Post.* posterior bone in the palato-quadrato; *Pect.* pectoral fin spine; *Phar.Br. II*, the pharyngo-branchial of the second arch; *Sc.* scapula; *Sup.Or.C.* supra-orbital canal; *V.L.L.* ventral lateral-line.

Fig 1 Pect LL InfOrC LSupOrC MandAnt



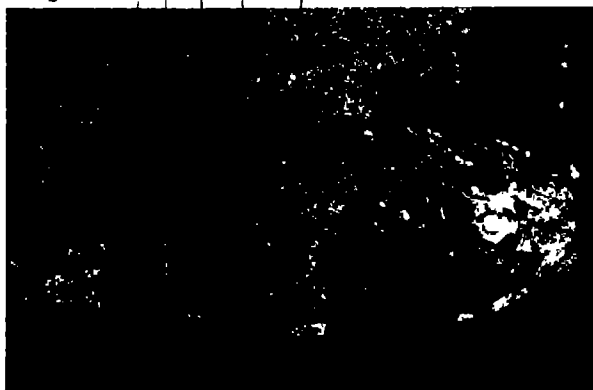
Sc OpC POpC PQPost Hy Gill R PQAnt
Mand Post Mand Spl

Fig 2 SupOrC LL Sc



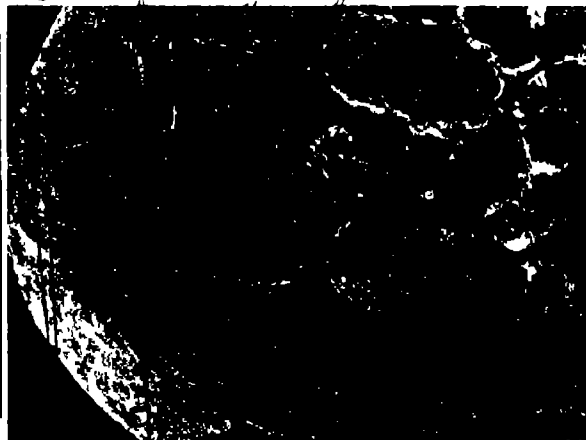
InfOrC Gu Mand Spl

Fig 3 BrIV III II I LL



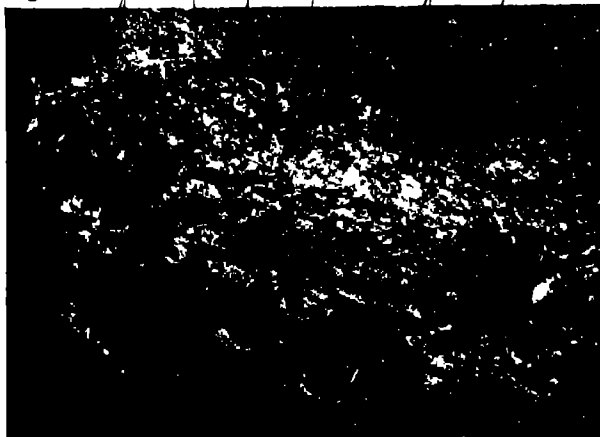
Sc OpC EpHy Mand Ray PQPost Mand

Fig 4 SupOrC InfOrC LL



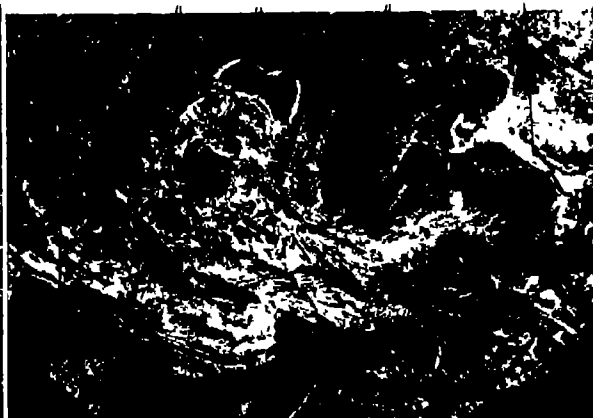
Mand Ant Mand Spl Mand Ray Mand Post OpC Sc Cor

Fig 5 SupOrC PQOt Ear PharBrll LL OpC



PQPost EpHy BrI II III IV Pect Centr
OpC

Fig 6 SupOrC Ear LL Sc



Eye Mand Spl OpC VLL

III—THE HYDATIDS OF MORGAGNI UNDER NORMAL AND EXPERIMENTAL CONDITIONS

By S. ZUCKERMAN* AND P. L. KROHN

From the Department of Human Anatomy, Oxford

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1—INTRODUCTION

Anatomical investigations of the appendages attached to the testis and epididymis began with enquiries into the origin of the fluid found in hydrocoeles. MORGAGNI (1682–1771), who was the first to draw attention to these minute structures, was opposed to the hypothesis that the tunica vaginalis is a secreting membrane. In place of that view, he advocated the opinion (1769) that the fluid is derived from ruptured testicular and epididymal hydatids.

The several investigations which followed MORGAGNI's observations showed clearly that his designation of these bodies as hydatids is fundamentally incorrect. Partly because of alternative descriptions advanced by subsequent workers, and partly because the subject has not been seriously considered since the earlier years of the present century, textbook descriptions of the testicular and epididymal appendages vary greatly with respect to the number, nomenclature, topography and embryological

* Beit Memorial Research Fellow.

significance of these bodies. The following synthesis of available views regarding the hydatids in man provides a necessary background to the consideration of the corresponding structures in monkeys, from which the material for the experimental work recorded in this paper was obtained.

2—THE HYDATIDS OF MORGAGNI IN MAN

The appendages of the human testis and epididymis comprise (a) the so-called hydatids found on the upper pole of the testis and on the head of the epididymis, (b) certain occasional appendages of the rete testis and vasa efferentia, (c) the ductuli aberrantes (usually associated with the name of HALLER) and (d) the paradidymis. In addition to these, EBERTH (1904) refers to the occasional presence, in the tunica vaginalis, (a) of chromaffin tissue, (b) of ectodermal tissue which occurs in relation to vestiges of the Wolffian body found in the spermatic cord and on the epididymis, and (c) of some small vesicles derived from the serous tunic of the testis and epididymis. Diversity in description applies mainly to the appendages found on the upper pole of the testis and the head of the epididymis.

The paradidymis, or organ of Giraldés, which is situated on the spermatic cord immediately above the head of the epididymis,* is generally agreed to be homologous with the paroophoron, and to be representative of blind caudal mesonephric tubules. There is also general agreement about the ductuli aberrantes, which vary in number, but of which one, situated near the junction of the epididymis and the ductus deferens, is fairly constant. They are generally stated to represent mesonephric tubules, or the fused collecting parts of such tubules (FELIX 1912), which have effected a connexion with the Wolffian duct.

The confusion about the appendages associated with the name of MORGAGNI mainly concerns the question of their separate identity. LUSCHKA (1854) first suggested that a pedunculated type of hydatid should be distinguished from a sessile kind. A sessile hydatid is usually found on the upper pole of the testis immediately below the head of the epididymis; two such bodies may occasionally be present in that situation. The position of the sessile hydatid (or hydatids) is not, however, constant, since similar hydatids may be found attached to the head of the epididymis. The pedunculated hydatid is invariably described as being attached to the head of the epididymis. It is not always single; as many as four such bodies have been found, attached close together on the head of the epididymis. A sessile hydatid is of more frequent occurrence than a pedunculated appendage. Thus it was present in ninety-three, and the pedunculated form only in twenty-nine, of 105 testes studied by TOLDT (1891).

The so-called sessile hydatid is variable in size and appearance. It is usually red owing to a rich blood supply, and it consists of loose connective tissue covered by ciliated columnar epithelium. Numerous tubular ingrowths of its surface epithelium

* FELIX (1912) makes the unusual statement that the paradidymis is situated between the testis and epididymis.

make it appear a somewhat glandular body. PIERSON (1930) describes the surface as "dentated", and as suggesting "the fimbriated end of the oviduct in miniature". Not unusually the appendage contains a small duct whose lining epithelium of ciliated columnar cells may either be flat, or thrown into extensive folds. The duct is variable in its development. Usually it comprises only a small blind cyst, distension of which may flatten its lining epithelium. In rare instances it is relatively long and tortuous, and may open on the surface of the hydatid; even more rarely it may pass back through the pedicle of the hydatid to run laterally to, and as far caudally as the middle of, the body of the epididymis. As a rule, the duct, when present, ends either within or at the base of the pedicle.

The pedunculated hydatids characteristically appear in adults as small vesicles of inconstant shape attached by pedicles of varying length to the head of the epididymis. The vesicle is usually stated to be covered externally by a flattened, and internally by a columnar epithelium. It is also said that the pedicle, which may be as long as 10 mm., is never canalized.

The embryological derivation of the sessile hydatid from the Müllerian duct has been thoroughly established. This view was originally suggested by KOBELT in 1857, and is strongly supported by the evidence brought forward by WALDEYER (1877), by LÖWE (1879), by ROTH (1880) and by TOLDT (1891). On the other hand, there is little agreement about the embryological significance of the pedunculated hydatids (appendices epididymis). Certain authors, for example KOBELT, regard them as isolated, blind, mesonephric tubules. ROTH suggests that they represent the peritoneal funnels of pronephric tubules. WATSON (1902), again, states that they are derivatives of either the mesonephros or the anterior end of the Wolffian duct. None of these suggestions, however, is supported by adequate embryological evidence.

TOLDT has advanced an alternative view that the pedunculated hydatids are derived, like those that are sessile, from the cranial end of the Müllerian duct. According to him, they develop, in the manner first described by MIHALKOVICS (1885), as small excrescences of the funnel-like opening of the duct. Their development is intimately linked with that of the sessile hydatid, which in turn is derived from the funnel-like opening itself. TOLDT's view, which is accepted by EBERTH (1904), is well supported by the following facts. There are numerous "intermediate" forms between typical sessile and typical pedunculated hydatids. Indeed on the basis of their investigation of fifty-three testes, WRIGHT and BROWN (1912) declare that the histological structure of even typical specimens of the two classes of hydatid is identical. Furthermore, it is known that the degree to which a hydatid is sessile or pedunculated varies and that sometimes only pedunculated appendages can be found. The distinction between the testicular and epididymal appendages appears, in short, to be only a topographical one.

In earlier embryonic life the Müllerian duct always ends on the upper pole of the epididymis, where it develops as the "sessile appendage", and where it may bud off

one or more "pedunculated" hydatids (TOLDT). The "sessile" hydatid gradually moves downwards with the growth of the head of the epididymis, until it is eventually attached, as a rule, in the interval between the epididymis and the testis. The pedunculated hydatid, which has already budded off from the Müllerian duct, is not affected by the caudal shift of the sessile hydatid. Where more than one pedunculated hydatid is found—apparently a frequent occurrence in embryos—their ducts often communicate with each other (EBERTH). It may be noted that the caudal shift of the sessile hydatid begins about the sixth or seventh month of embryonic life (TOLDT), and that at this stage a part of the Müllerian duct, which may still be patent, is usually to be recognized on the lateral side of the body of the epididymis. It may also be noted that the most rapid rate of growth of the hydatids occurs in the first years of life, and that between the fourth and tenth years the appendages are at their maximum size. Their variable shape during maturity and later life is due, according to TOLDT, not to continued growth but to variable degrees of distension occasioned by products of secretion.

The earlier investigators of the hydatids—LUSCHKA, LEWIN,* BECKER* and ROTH—described the occasional passage through the sessile hydatid of a vas aberrans which began either in the rete testis, in the vasa efferentia, or in a lobule of the epididymis. They also frequently reported the discovery of sperms within a duct—presumably such a vas aberrans—which opened on the surface of the hydatid. When fully patent, vasa aberrantia, which are generally believed to represent the remains of mesonephric tubules, permit the passage of spermatozoa, and as the ducts sometimes end blindly in the region of the head of the epididymis, they provide an anatomical basis for the development of sperm cysts in that region. ROTH describes several instances of ducts passing either from the head of the epididymis or from a vas efferens into a sessile hydatid, and suggests that the occasional presence of sperms in the fluid of the tunica vaginalis is due to their passage through these ducts. ROTH also points out that the ducts occasionally end blindly in sessile hydatids, which they can, by their distension, transform into sperm cysts. He also remarks on the occasional passage of two ducts through the same hydatid—the one a vas aberrans of the type described above, and the other the vestige of the Müllerian duct. Two such ducts have never been described as communicating. RICHMOND (1883) also refers to the combination in the sessile hydatid of Müllerian and Wolffian rudiments, stating that a minute fibrous body which is sometimes attached to the sessile hydatid is a derivative of the Wolffian body.

Considerable doubt has been cast on these observations by the failure of successive investigators to confirm them. WALDEYER (1877), for example, refers to his failure to demonstrate in any testis a connexion between a sessile hydatid and a seminiferous tubule. TOLDT again, whose material comprised 132 testes, also records his failure to demonstrate such a connexion, or to find spermatozoa in any duct or cyst within the

* Quoted by TOLDT.

sessile hydatid. GRIFFITHS (1893) also failed to find such a connexion, and he declares that all small spermatozoa-containing cysts originate in dilatations of the tubules of the coni vasculosi, and that all large cysts of similar character develop as dilatations of vasa efferentia.

We have discussed this matter somewhat fully, since in one of the specimens we ourselves examined (O.M. 135 B, p. 158) there undoubtedly was a connexion between a tubule of the epididymis and a hydatid whose form was typical of those that are derived from the cranial end of the Müllerian duct. The difficulty of explaining a communication between derivatives of the Müllerian and Wolffian ducts was fully realized by ROTH. According to him, however, such an intercommunicating vas aberrans is occasionally present in the female, and connects a fimbria of the Fallopian tube with the epoöphoron.* In spite of the fact that in normal development the Wolffian duct never communicates with the coelomic cavity, ROTH, following WALDEYER (1877), therefore found it necessary to postulate that the Wolffian and Müllerian ducts can form a secondary communication at their cranial ends—such as occurs normally in selachians.

The observations we have considered above may be summarized as follows. Usually one, occasionally two, and rarely more, minute appendages are attached to either the upper pole of the testis, to the tissue filling the interval between the testis and the head of the epididymis, or to the head of the epididymis. These structures are usually sessile, but may occasionally be pedunculated. Their form varies from minute cysts, lined by ciliated columnar epithelium, to small bodies closely resembling a miniature fimbriated end of a Fallopian tube. Every form of hydatid which falls under this general description is in all likelihood derived from the cranial end of the Müllerian duct. Occasionally the hydatids may be traversed by a duct, arising from either the head of the epididymis or from the vasa efferentia, which is embryologically related to the Wolffian system.

3—THE HYDATIDS OF MORGAGNI IN MONKEYS

Little is known of the appendages of the testis and epididymis in mammals other than man. GRIFFITHS (1893) states that he found a hydatid of Morgagni only in the horse, but he does not mention the number of types he investigated. CURLING (1852) compares the sessile hydatid with "the remarkable omental processes attached to the superior part of the testicle in the Rodentia and other animals". The literature apparently does not contain any mention of the occurrence of these structures in subhuman primates. None, for example, occurs in SONNTAG's work on the morphology of the apes (1924) or in the volume on the anatomy of the rhesus monkey edited by HARTMAN and STRAUS (1933).

* Called by ROTH "paroöphoron". His figures make it clear that he meant the epoöphoron.

The material available for investigation in the present study comprised the testes of:

- One langur (*Presbytis entellus*).
- One green monkey (*Cercopithecus aethiops sabaeus*).
- Eighty-two rhesus macaques (*Macaca mulatta*).
- Two pig-tailed macaques (*Macaca nemestrina*).
- One common macaque (*Macaca irus*).
- One mandrill (*Mandrillus sphinx*).
- One drill (*Mandrillus leucophaeus*).
- One guinea baboon (*Papio papio*).
- One common marmoset (*Hapale jacchus*).

Testicular and epididymal appendages (hydatids of Morgagni) were found in all nine species investigated.

Certain of the testes studied were recovered from animals that had not been subjected to any experimental procedures connected with the physiology of the reproductive organs. The remaining specimens were taken from animals which for varying periods previous to autopsy had been injected with sex hormones or related substances. The primary purpose of these injections, which are indicated in Table I, was the investigation of the endocrine control of the prostate.

TABLE I—LIST OF ANIMALS WHOSE HYDATIDS OF MORGAGNI WERE STUDIED. THE FIGURES GIVE THE NUMBER OF NORMAL SPECIMENS, AND SPECIMENS WHICH HAD BEEN INJECTED WITH THE SUBSTANCES NOTED IN THE COLUMN HEADS

Species	Normal	Oestrone	Male hormone	Progestosterone	Extract of anterior pituitary	Oestrone and male hormone	Oestrone and progestosterone	Oestrone and cholesterol	Oestrone and epicholesterol
<i>Presbytis entellus</i>	—	1	—	—	—	—	—	—	—
<i>Cercopithecus aethiops sabaeus</i>	1	—	—	—	—	—	—	—	—
<i>Macaca mulatta</i>	17	17	3	2	3	20	12	6	2
<i>Macaca nemestrina</i>	2	—	—	—	—	—	—	—	—
<i>Macaca irus</i>	—	1	—	—	—	—	—	—	—
<i>Mandrillus sphinx</i>	1	—	—	—	—	—	—	—	—
<i>Mandrillus leucophaeus</i>	—	1	—	—	—	—	—	—	—
<i>Papio papio</i>	1	—	—	—	—	—	—	—	—
<i>Hapale jacchus</i>	1	—	—	—	—	—	—	—	—

The hydatids of the langur, one of the pig-tailed macaques, the mandrill, the marmoset, and thirty-one of the rhesus monkeys were studied under the dissecting microscope. Those of the langur, the green monkey, the two pig-tailed macaques, the common macaque, the drill, the Guinea baboon, and sixty-one rhesus monkeys were studied histologically. After fixation in Bouin's fluid, the hydatids, together with a piece of testis and epididymis, were removed and serially sectioned at 10 μ . Most of the sections were stained with haemalum and eosin. A representative series was also stained with iron haematoxylin and van Gieson. Like the specimens studied

histologically, those examined under the dissecting microscope had been fixed in Bouin's fluid.

A paradidymis was not seen in any specimen, but as no special steps were taken to demonstrate it, our failure to do so does not imply that it is invariably absent. Ductuli aberrantes were not sought for, and the only appendages seen were those which come under the definition of hydatids of Morgagni.

The Rhesus Monkey (Macaca mulatta)

(a) Macroscopic Observations.

Hydatids are found attached to the head and to the body of the epididymis (179 R, fig. 1, Plate 15), to the serosa between the head of the testis and the epididymis (148 R, fig. 1, Plate 15), and to the testis itself (151 R, fig. 1, Plate 15). The way they are attached provides no basis for differentiating between epididymal and testicular appendages, for the degree to which hydatids are sessile or pedunculated varies considerably, and extremes of both types are found attached to both the testis and the epididymis. On this point our histological observations are fully in accord with those made with the help of the dissecting microscope. It is also idle to try to differentiate the hydatids on the basis of their sites of attachment, since precisely the same types of appendage, both in general form and histological structure, are found on the testis and the epididymis.

When present on the testis, the hydatids are almost invariably attached to its upper pole, and often in relation to the fold of serosa that constitutes the superior ligament of the epididymis. Sometimes the pedicle of a hydatid attached in this region passes into what appears to be a duct connecting the testis and the head of the epididymis (170 R, 148 R, fig. 1, Plate 15). Histological examination generally failed to show that this "duct" is canalized. Appendages of the epididymis are almost always attached to the inferior margin of its head and to its antero-lateral border. Under the dissecting microscope a slightly raised ridge, with which the pedicle of a hydatid may be continuous, is sometimes seen on the extreme lateral margin of the body of the epididymis (163 R, 179 R, fig. 1, Plate 15). This ridge evidently represents the obliterated Müllerian duct. Its histological character is considered in a later section.

The most usual point of attachment of an epididymal appendage is the region of the junction between the head and body of the epididymis, where the "Müllerian ridge", when present, usually ends (179 R, fig. 1, Plate 15). The second most frequent point of attachment is the lower end of the body, at the caudal end of the ridge (170 R, fig. 1, Plate 15). Occasionally vesicular swellings can be seen on the presumed obliterated duct (163 R, 179 R, fig. 1, Plate 15); these vesicles may be raised to form small pedunculated hydatids. Hydatids also occur on the antero-inferior margin of the head of the epididymis. Very rarely they may be found on the testicular surface of either the head or the upper part of the body (163 L), such appendages projecting

into the sinus of the epididymis which, too, is very inconstant in its appearance (fig. 5, Plate 16).

As a general rule only one hydatid is present on each testis and epididymis. Not more than two were seen on any of the present specimens. There was no trace of a hydatid on eleven of the sixty testes examined under the dissecting microscope, and in all, fifty-nine hydatids were found in this series of testes. Thirty-eight of these were attached to the epididymis, and twenty-one to the testis or to the superior ligament of the epididymis. When present on both testes of a single animal, hydatids are occasionally attached in corresponding places.

The hydatids vary in form from small knob-like bodies to relatively large foliated structures of completely irregular shape. Their pedicles are very variable in length, and in size the hydatids range according to the dimensions given in Tables III, IV, and V. As will be shown later, those hydatids removed from animals which had been injected with oestrone are generally larger than those removed from normal monkeys, a difference which is sometimes obvious to direct observation. The only testes on which hydatids were not found under the dissecting microscope were those of uninjected animals. It is not unlikely that hydatids, which normally would be invisible under a dissecting microscope, become large enough to be seen after stimulation with oestrone.

It is usually stated that the hydatids found in man decrease in size with age. Whether or not similar age changes occur in the rhesus monkey could not be determined with the available material.

The following hydatids are of special interest:

O.M. 151. Right testis (151 R, fig. 1, Plate 15)—Two hydatids, the one somewhat larger than the other, were attached to the upper pole of the testis slightly in front of the head of the epididymis. The appendages, which were 3 mm. apart, had short pedicles, that of the larger emerging from what might be termed a hilum (cf. 170 R, fig. 1, Plate 15). The pedicles were duct-like in appearance, and passed into a raised tubular ridge on the testis. The ridge extended on either side beyond the attachment of the hydatid, and blended with the serosa covering the tunica albuginea. Histological examination showed that this ridge was not canalized. The larger and fimbriated hydatid was divided into three fairly separate lobules by relatively deep clefts, and its surface was pitted by the openings of numerous glands.

Two hydatids were present on the left testis, but only one was attached to the testis itself, the second appendage being connected to the epididymis at the junction of the body and the tail.

O.M. 163. Left testis—Towards the lateral side of the inferior surface of the head of the epididymis, under which it is completely hidden from view, was a large bilobed sessile hydatid with serrated margins and a pitted surface (fig. 5, Plate 16).

O.M. 179. Right testis—Two knob-like slightly pedunculated hydatids were attached to the antero-lateral border of the epididymis, the one at the junction of the body and

head, the other at the junction of the body and tail. In between the two was to be seen the apparent remains of an obliterated duct. A canal which may have been the remains of the Müllerian duct was actually present in this ridge (see below).

(b) *Microscopic Observations.*

As in man, the Müllerian duct may be represented in the male rhesus monkey by appendages which vary from simple tags of fibro-muscular tissue to bodies with a complex epithelial structure. In order to simplify their histological description we have therefore subdivided the hydatids into four types.

Type I—The first type comprises small tongues of fibro-muscular tissue covered by flattened epithelium that is continuous with the serosa covering the testis and epididymis.

Type II—Type II comprises small bodies which in general structure are similar to those defined as Type I, but which enclose a central duct lined by columnar epithelium.

Type III—This type of appendage is composed of loose fibro-muscular tissue covered by ciliated columnar epithelium. A definite central duct is not enclosed within this type of hydatid.

Type IV—The fourth type of hydatid is similar in structure to the third, and in addition encloses a central duct of variable complexity.

The relative frequency of these types of appendage is indicated in Table II. In this table the specimens are separately grouped according to the experimental treatment given the animals from which they were recovered.

TABLE II—THE DIFFERENT TYPES OF HYDATID OF MORGAGNI IN THE RHESUS MONKEY AS DETERMINED FROM SERIAL SECTIONS. THE SPECIMENS ARE SEPARATELY GROUPED ACCORDING TO THE EXPERIMENTAL TREATMENT GIVEN THE ANIMALS FROM WHICH THEY WERE RECOVERED.

Type I—Fibro-muscular tags.

Type II—Fibro-muscular bodies containing a central cyst lined by columnar cells.

Type III—Fibro-muscular bodies covered by columnar epithelium.

Type IV—Similar to Type III, but containing a central cyst lined by columnar epithelium.

	Types				Total
	I	II	III	IV	
Normal	2	0	9	3	14
Oestrone	1	1	13	10	25
Oestrone and delay*	0	0	1	2	3
Male hormone	0	0	3	1	4
Progesterone	0	0	2	1	3
Anterior pituitary	0	0	3	3	6
Oestrone and male hormone	0	5	8	9	22
Oestrone and progesterone	2	6	9	4	21
Oestrone and cholesterol	0	0	5	3	8
Oestrone and epicholesterol	0	0	2	0	2
Totals	5	12	55	36	108

* Animals autopsied 11 and 20 days after the cessation of a course of oestrone injections.

Type I—Little need be said about the structure of appendages of this kind beyond the fact that they comprise minute tag-like condensations of fibro-muscular tissue whose covering of flattened epithelial cells, which is continuous with the visceral layer of the tunica vaginalis, sometimes appears to be deficient.

Type II—Hydatids of this type are usually attached to the antero-lateral margin of the epididymis. When present in conjunction with an appendage belonging to Type III or Type IV they are more caudally disposed on the epididymis.

As a rule they comprise fairly close condensations of fibro-muscular tissue, which is irregularly covered by flattened epithelial cells, and which encloses a blind duct lined by columnar epithelium (figs. 3 and 4, Plate 16). The muscle fibres immediately surrounding the duct are usually circularly disposed. The duct is lined by a single layer of columnar epithelium which is set on a well-defined basement membrane. In many sections the cells lining the duct appear to be ciliated. Usually too, the duct epithelium is regularly disposed, but in two specimens (O.M. 42 C and 160 R) it was folded in a manner reminiscent of the epithelial foldings of the Fallopian tube. The "duct" is often merely a blind cyst containing some secretion, and in such cases its epithelium may be flattened. Both the epithelium of the central duct and the muscle fibres of this type of hydatid respond in the same way to the injection of oestrone as do the hydatids of Types III and IV (see below).

There can be little doubt that epididymal appendages of the above kind are persistent parts of the Müllerian duct itself. Thus the vesicular swellings of the ridge found on the antero-lateral border of the epididymis are invariably of this form. Fig. 4, Plate 16, for example, is a photomicrograph of the swelling seen on the lower part of the antero-lateral border of the epididymis of O.M. 163 R, fig. 1, Plate 15. Similar structures were seen on both sides in O.M. 161. The "Müllerian" ridge itself is rarely canalized. O.M. 179 R, fig. 1, Plate 15, shows a testis with a small fimbriated appendage on the upper part of the lateral border of the epididymis, and a vesicular swelling some distance below it. The upper hydatid has a relatively long non-canalized pedicle, and in form it is similar to hydatids of Type III. The lower swelling is merely a blind duct 0.5 mm. long and 0.2 mm. in widest diameter. The ridge intervening between the upper hydatid and the vesicular swelling was also sectioned but a properly differentiated duct was not found within it. It does, however, contain a vertical channel of minute diameter, blind at both ends, and some 0.5 mm. in length. This duct is lined by irregularly disposed cuboidal epithelium, which in places is wanting. It neither contains any blood cells nor communicates with any obvious vascular or lymphatic channels. While it is impossible to decide with certainty about the identity of this channel, there is a strong likelihood that it also represents part of the Müllerian duct.

In only one specimen, O.M. 151 R, fig. 1, Plate 15, was an appendage of Type II not in relation to the antero-lateral border of the epididymis. The macroscopic appearance of this testis has been detailed above (see p. 154). The smaller of the two hydatids

on this testis proved to be a hollow fibro-muscular body covered only by flattened serous epithelium. The fibrous ridge to which its pedicle was attached is not canalized. The second hydatid was also attached to this ridge, and is of the kind referred to as Type III.

The tubular structure to which testicular hydatids are sometimes connected (O.M. 148R, O.M. 170R, fig. 1, Plate 15), and which generally passes in the superior epididymal ligament between the testis and the epididymis (see p. 153), was found to be canalized in only one specimen, O.M. 160R. Fig. 2, Plate 16, shows the hydatid in question attached to a fibrous band (superior epididymal ligament) passing between the testis and the head of the epididymis. In this band is a blind cyst, 0.8 mm. long and 0.5 mm. in diameter, whose epithelium is slightly folded. A few millimetres distal to this cyst there is a second cyst, similar in size and structure, lying in the fibrous tissue between the testis and the epididymis (fig. 3, Plate 16).

Type III—Most testicular and epididymal appendages are of the kind defined as Type III. An appendage which comes under this definition has a non-canalized pedicle of variable length which is covered by a flattened epithelium that is continuous with the serosa of either the testis or the epididymis, depending on its site of attachment. The hydatid itself is usually leaf-like in section, and comprises a vascular fibro-muscular stroma covered by columnar epithelium (figs. 8, 9, and 10, Plate 17) that is sharply differentiated from the flat epithelium of the pedicle (fig. 16, Plate 18). The separate elements of the stroma are relatively widely dispersed in monkeys that have not been injected with oestrone, and the vascular channels, which can frequently be traced to the base of the surface epithelium (fig. 20, Plate 19), are conspicuous and widely dilated. The surface epithelium consists of a moderately high and sometimes irregular layer of columnar cells set upon a definite basement membrane. The nuclei are relatively large (figs. 17 and 18, Plate 19), and in many sections the glandular and surface cells appear ciliated (cf. fig. 19, Plate 19). Mitotic figures are rarely present in the epithelium of hydatids removed from normal control animals. In many specimens the surface epithelium dips into the stroma to form tubular glands of varying depth (fig. 12, Plate 17), but like the surface epithelium, the glandular cells are rarely found to be secreting in hydatids recovered from normal control animals.

Type IV—The thirty-six hydatids of this type have the same general character as Type III, but in addition they enclose a central duct. The epithelium of the duct forms an even layer in thirty of the thirty-six specimens, and is thrown into folds reminiscent of those of the Fallopian tube in the remaining six (fig. 14, Plate 18). In three of the latter six, and four of the thirty, the duct passes completely through the hydatid to open into the sac of the tunica vaginalis (fig. 11, Plate 17; fig. 16, Plate 18). The duct frequently penetrates deeply into the pedicle (cf. figs. 13 and 15, Plate 18), and in a few cases reaches into epididymal tissue beyond the point of pedicular attachment.

In two specimens (O.M. 124 and O.M. 144), two separate central ducts were found in a single hydatid, one duct being confined to the pedicle, and the second to the body of the hydatid. Presumably both represent separated parts of the embryonic Müllerian duct. Sometimes the central duct is much coiled and bent. In one specimen, O.M. 143A, the two ends of a blind duct almost meet to form a tubular ring. In two other specimens (O.M. 23 and O.M. 36) the hydatid contains a solid mass of epithelial cells—presumably derived from the epithelium of an obliterated part of the Müllerian duct. In one of these specimens (O.M. 23) a patent central duct is also present.

As a general rule, and almost always when the duct is well developed, the duct epithelium is surrounded by a condensation of circularly disposed muscle fibres. The epithelium of the duct is composed of columnar cells, between which leucocytes may occasionally be seen passing. In specimens that have not been subjected to the influence of oestrone, little secretion is present, and the nuclei are relatively large. A delicate vascular stroma supports the duct epithelium when it is thrown into folds. The resemblance of a hydatid to a minute Fallopian tube can be very close, as for example M.M. 18, fig. 14, Plate 18, in which a well-differentiated duct ends by traversing a cap of fibro-muscular tissue that is covered by ciliated columnar epithelium.

The following three hydatids present unusual characters:

O.M. 109B—The blind central duct of this specimen extends back through the pedicle and ends deeply in the midst of lobules of the epididymis.

O.M. 23A—This hydatid contains a blind central cyst which is situated between lobules of the epididymis, and which extends slightly into the pedicle. The pedicle also contains a solid mass of epithelial cells that are presumably derived from the obliterated Müllerian duct. Some of these cells are disposed in the form of non-canalized tubular glands.

O.M. 135B—As noted earlier on, a duct of Mullerian origin which opens on the surface of a human hydatid of Morgagni may on rare occasions communicate with a seminiferous tubule. The hydatid found on the left testis of O.M. 135 presented a similar anomaly.

The hydatid is of the kind described as Type IV, and comprises a fibro-muscular stroma with an indented surface covered by high columnar epithelium. The base of the hydatid is attached to a relatively large cyst formed by the expansion of one end of a highly convoluted epididymal tubule (fig. 25, Plate 20). This epididymal tubule does not communicate with the vas deferens, and by definition it must be regarded as a vas aberrans of the head of the epididymis.

Passing through the hydatid is a duct which at its one extremity opens on the surface of the hydatid into the tunica vaginalis, and at its other opens into the cyst of

the vas aberrans. In order to study these relations tracings were made of projections of each section of the serially sectioned specimen.

The aberrant conus vasculosus is 2.31 mm. in greatest diameter (fig. 22, Plate 20). The maximum diameter of the ovoid cyst it contains is 2.09 mm., its transverse diameter being 1.6 mm. The tubule of the abnormal conus vasculosus cannot be differentiated under the low power of the microscope from that of any normal epididymal lobule. Its epithelium is composed of moderately high columnar cells which are set upon a definite basement membrane and which have basally disposed nuclei. Under the high power of the microscope, however, the epithelium of the vas aberrans can be distinguished from that of normal epididymal tissue by the diffuseness of its inner borders, a change which was probably due to the greater pressure that existed within the aberrant lobule. The epithelium of the cyst is in general somewhat flattened.

The cyst becomes somewhat abruptly constricted about 0.5 mm. from the pole opposite the entry of the vas aberrans. Its muscular wall in the region of constriction is markedly thicker than elsewhere, and its epithelium is unusually high, and more obviously ciliated. In most places the epithelium in this region is either heaped or thrown into folds (fig. 26, Plate 20). Stromal tissue extends only into the proximal parts of the folds, whose free ends consist of cells lying end to end and separated only by opposed basement membranes.

The interior of the large cyst is empty; whatever material it might once have contained would have been washed out, however, during the process of histological preparation.

Lying lateral to the distal constricted part of the cyst are two much smaller intercommunicating blind cysts, each containing several leucocytes (fig. 26, Plate 20). These smaller cysts have a maximum diameter of 0.25 mm., and neither communicates with the main cyst from which they presumably budded. Their epithelium is cuboidal.

The general structure of the hydatid itself does not require special description. The tall ciliated columnar epithelium of its central duct is continuous both with that covering the surface of the hydatid (fig. 23, Plate 20) and with that lining the epididymal cyst (fig. 25, Plate 20). The duct makes a right-angle bend before it opens on the surface of the hydatid.

The central duct is identical in appearance with those that usually end blindly within a hydatid, and there is no reason to question its Müllerian origin. The epididymal nature of the structure which has been described as a vas aberrans is equally plain, and consequently it is necessary to draw the conclusion that in this specimen structures derived from the Müllerian and Wolffian ducts respectively communicate with each other. Available data provide no safe ground for speculating on the phylogenetic significance of such a communication, and in the circumstances the present abnormality should be regarded, as WALDEYER and ROTH regarded corresponding human anomalies, as a secondary communication between the Müllerian and Wolffian ducts.

*Species other than Macaca mulatta**(a) Macroscopic Observations.*

The hydatids found in species of monkey other than the rhesus macaque (*Macaca mulatta*) appear to have the same topographical relations, general form, and histology. They are accordingly only briefly described.

O.M. 82. *Presbytis entellus*, Hanuman langur. Immature animal. O.M. 82R, fig. 1, Plate 15—A faintly marked ridge is present on the antero-lateral margin of the body of the epididymis. The "Müllerian" ridge ends below at the junction of the body and tail of the epididymis, and at its termination is attached a flat and oval hydatid with serrated margins. A corresponding hydatid is present on the opposite testis, to the upper pole of which a second appendage is connected by a long pedicle.

P. 5. *Macaca nemestrina*, pig-tailed macaque. Mature animal—Attached laterally to the lower margin of the head of the epididymis is a small wart-like hydatid which is divided by clefts into numerous folds. The base of the hydatid is attached to a ridge which seems to represent an obliterated duct, the ridge continuing caudally in the antero-lateral border of the epididymis, and medially along the inferior margin of the head of the epididymis. Microscopic examination showed that the ridge is not canalized. A somewhat similar hydatid is present on the opposite side.

Mandrillus sphinx, mandrill. Mature animal—A thin foliated hydatid is attached by a long pedicle to the superior ligament of the epididymis. Two somewhat hemispherical excrescences are present on the testis in the region where the pedicle is connected. A smaller but somewhat similar hydatid is present on the opposite side.

O.M. 51. *Hapale jacchus*, common marmoset. Mature animal—On the left side a relatively large and flattened sessile hydatid is attached by a broad base to the superior epididymal ligament, through which it gains attachment to both the testis and the lower part of the head of the epididymis. Two hydatids are present on the opposite side, one being attached to the testis, the other to the epididymis.

(b) Microscopic Observations.

O.M. 82. *Presbytis entellus*, Hanuman langur. Immature animal—The hydatid shown on the lower part of the epididymis of O.M. 82R, fig. 1, Plate 15, is similar in structure to Type IV of the rhesus monkey. It encloses both a minute central duct which opens on its surface (fig. 6, Plate 16), and a blind cyst which is situated more deeply, close to the point where the pedicle of the hydatid is attached to the "Müllerian ridge". About 1 mm. cranial to the point of attachment of the hydatid, the ridge contains a blind duct 0.23 mm. long, which is lined by cuboidal cells (fig. 7, Plate 16). The two deeply lying ducts neither communicate with each other nor with the duct that opens into the sac of the tunica vaginalis; there cannot, however, be any doubt that they all represent parts of the Müllerian duct.

O.M. 35. Cercopithecus aethiops sabaes, green monkey. Immature animal—A large hydatid was attached to the tunica of the testis, and in general character it is the same as the fourth type of rhesus hydatid. There are fewer indentations of the surface epithelium, but the hydatid contains a central duct which passes from its pedicle and opens on its surface into the sac of the tunica vaginalis. The duct bent abruptly on itself before passing to the surface (fig. 11, Plate 17).

P. 6. Macaca nemestrina, pig-tailed macaque. Mature animal—On the right side a large hydatid, divided by deep clefts into several lobes, was attached to the fibrous tissue between the testis and the head of the epididymis. On the left a similar appendage was connected by a long pedicle to the epididymis. The left hydatid contains a central duct, lined by regularly disposed epithelium which opens into the sac of the tunica vaginalis. The inactive low columnar epithelium covering this hydatid is shown in fig. 17, Plate 19.

O.M. 34. Macaca irus, common macaque. Immature animal—No hydatid was found on the right testis. A small appendage similar to the third type of rhesus hydatid was attached in the angle between the left testis and epididymis.

M.M. 22. Papio papio, Guinea baboon. Immature animal—A single hydatid was attached to the antero-lateral margin of the head of the epididymis. In general character it is the same as the fourth type of rhesus hydatid. The central duct is blind, and its epithelium is folded.

O.M. 114. Mandrillus leucophaeus, drill. Immature animal—A single hydatid was attached on each side in the angle between the testis and the head of the epididymis. Both hydatids contain a large central duct lined by folded epithelium, which is separated by a distinct basement membrane from a condensation of circularly disposed muscle fibres. On the right side the duct opens into the sac of the tunica vaginalis.

4—THE EFFECT OF SEX HORMONES ON THE HYDATIDS OF MORGAGNI

The thickness of each of the 108 hydatids that were serially cut was estimated from the number of sections in which it appeared; the greatest cross-sectional area of each hydatid was calculated from projections on to squared paper. The figures obtained, as well as their products, provide rough measures of the sizes of the hydatids in the various experimental groups studied. The hydatids are arranged in the following tables according to age, the ages of the animals being estimated on the basis of SCHULTZ's figures for weight-age and dentition-age relationships in the rhesus monkey (1933, 1935).

(a) *Gonadotropic extract of Anterior Pituitary*

Six hydatids were recovered from three animals that had been injected with 20 mg. of anterior pituitary extract daily, injections being continued for 24 days in the case

TABLE III—DIMENSIONS OF NORMAL HYDATIDS ARRANGED ACCORDING TO APPROXIMATE AGES OF ANIMALS FROM WHICH THEY WERE RECOVERED

No. of animal	Species	Weight (g.)	Age (months)	Thickness of hydatid (mm.) = <i>a</i>	Max. cross-sectional area (sq. mm.) = <i>b</i>	Size index = <i>ab</i>
O.M. 112	<i>Macaca mulatta</i>	1000	18	0.78	2.1	1.6
O.M. 118	"	1300	19	0.4	3.2	1.3
O.M. 38A	"	2360	24	0.29	6.5	1.9
O.M. 38B	"	2360	24		Minute	
O.M. 39A	"	2700	28		Minute	
O.M. 39B	"	2700	28		Minute	
O.M. 124B	"	2840	30	0.85	6.0	5.1
M.M. 27A	"	2700	32	0.46	3.9	1.8
M.M. 27B	"	2700	32	0.32	12.2	3.9
O.M. 163B	"	—	40	0.74	10.35	7.7
O.M. 89	"	4200	44	1.0	19.6	19.6
O.M. 90A	"	5000	50	0.5	4.2	2.1
O.M. 90B	"	5000	50	0.35	3.6	1.3
O.M. 20	"	6720	c. 120	1.64	78.3	128.0
M.M. 22	<i>Papio papio</i>	—	20	0.77	43.8	33.7
O.M. 35	<i>Cercopithecus aethiops sabaeus</i>	—	Immature	1.61	17.8	28.7
P. 6 A	<i>Macaca nemestrina</i>	—	Mature	2.38	58.5	139.2
P. 6 B	"	—	Mature	2.2	76.5	168.3

TABLE IV—DIMENSIONS OF HYDATIDS AFTER INJECTION OF GONADOTROPIC EXTRACT OF ANTERIOR PITUITARY, MALE HORMONE, OR PROGESTERONE, ARRANGED ACCORDING TO THE APPROXIMATE AGES OF THE ANIMALS STUDIED

No. of animal	Weight (g.)	Approx. age (months)	Substance injected	Amount of substance injected daily (mg.)	Days injected	Thickness of hydatid (mm.) = <i>a</i>	Max. cross-sectional area (sq. mm.) = <i>b</i>	Size index = <i>ab</i>
O.M. 81A	2480	22	Gonadotropic extract of anterior pituitary	20	24	0.52	5.0	2.6
O.M. 81B	2480	22		20	24	0.6	7.3	4.4
O.M. 66A	3060	23		20	15	1.41	139.3	196.4
O.M. 66B	3060	23		20	15	1.14	66.0	75.2
O.M. 65A	3260	32		20	15	0.45	4.6	2.1
O.M. 65B	3260	32		20	15	0.63	4.5	2.8
M.M. 24A	1800	20		10	10	0.38	14.3	5.4
M.M. 24B	1800	20		10	10	0.25	9.2	2.3
O.M. 67	2700	24		5	12	0.75	17.5	13.1
O.M. 47	2680	24		5	28	0.44	10.6	4.7
O.M. 52	3220	37	Progesterone hormone preparation	0.3	14	0.7	5.8	4.1
O.M. 48A	4560	48		0.5	14	0.57	6.6	3.8
O.M. 48B	4560	48		0.5	14	0.6	4.3	2.6

of one animal (O.M. 81) and for 15 days in the case of the other two (O.M. 65 and O.M. 66). The dimensions of the hydatids are given in Table IV. Those removed from O.M. 66 are relatively enormous, but on the available data this fact must be regarded as an individual anomaly, and in the main not an effect of the injections. The other hydatids are slightly larger than normal (Table III), but whether the slight difference is significant is doubtful.

Histologically the hydatids appear to be little, if at all, different from the normal. Those recovered from O.M. 65 and from O.M. 81, the animal which had received most of the hormone, show no definite distinguishing characteristics. Those of O.M. 66 contain large central ducts, and the epithelium of the right duct, which opens into the sac of the tunica vaginalis, is extensively folded. A few mitotic figures were seen in the epithelium of this duct, and one dividing cell was found in the surface epithelium. The nuclei of the more superficial stromal cells are somewhat swollen, and there is a layer of amorphous eosinophilic extracellular material beneath the surface epithelium. It is, however, unlikely that the individual characteristics of these two hydatids were the result of the anterior pituitary injections; the other four hydatids recovered from animals that had been similarly treated resemble those of O.M. 66 far less than they do the hydatids of normal animals.

(b) *Male Hormone*

The four hydatids removed from monkeys that had been injected with male hormone (M.M. 24, O.M. 67, O.M. 47) are not distinguishable from normal specimens. The dimensions of the hydatids are given in Table IV. While the wide variability in the dimensions of the normal appendages (Table III) precludes close comparison with those of other series, it is plain that the hydatids had not increased in size as a result of the injections. None of the specimens provides any evidence of active cellular growth in either the epithelial or stromal tissue. The latter is as vascular, and in general as sparsely packed, as in the normal specimen.

The columnar epithelium of a very folded central duct of one of these four hydatids (O.M. 67) is taller than normal. The duct also contains some secretion. As in the case of O.M. 66 (above), it is likely that this appearance of activity is a normal characteristic of well-differentiated central ducts, and that it does not result from endocrine treatment.

(c) *Progesterone*

Three hydatids were recovered from two animals that had been injected for 14 days with progesterone, the one with 0.3 mg. and the other with 0.5 mg. daily. The dimensions of these hydatids are given in Table IV; they are little bigger than the smaller of the normal hydatids. In general, too, their histological appearance is not different from the normal.

(d) *Oestrone*

Oestrone stimulates considerable growth in the hydatid,* as can be seen both in the obvious increase in size (Table V) and in the frequency of mitotic figures in histological preparations. Of the fourteen normal rhesus hydatids listed in Table III, only three have a size index greater than 6, while only two of the twenty hydatids from monkeys that had been injected with oestrone for a period of 14 days or more

* This fact was referred to elsewhere in connexion with a suggested interpretation of the changes induced by oestrogens in the male reproductive tract (ZUCKERMAN 1936a).

had a size index less than 6. The abnormally large hydatids found in two normal specimens, O.M. 20 and O.M. 89, raise the mean size index of the normal hydatids to 12.5. The mean size index of monkeys that had been injected with oestrone for 14 or more days was 24.5, and as noted above (p. 154) the greater size of the hydatids removed from animals that had been injected with oestrone was often apparent under the dissecting microscope. One animal, O.M. 124, was unilaterally castrated before it was subjected to a 14-day course of oestrone injections. The size index of the normal hydatid was 5.1, that of the hydatid which had been stimulated by oestrone, 20.2.

TABLE V—DIMENSIONS OF HYDATIDS AFTER INJECTIONS OF OESTRONE, ARRANGED ACCORDING TO THE TIME OESTRONE WAS ADMINISTERED

No. of animal	Species	Weight (g.)	Approx. age (months)	Amount of oestrone injected daily (mg.)	Days injected	Thick-ness of hydatid = a	Max. cross-sectional area (sq. mm.) = b	Size index = ab
M.M. 16	<i>Macaca mulatta</i>	3500	40	1	6	0.56	32	17.9
M.M. 20A	"	1800	15	5	6	0.22	2.25	0.5
M.M. 20B	"	1800	15	5	6	0.56	6.75	3.8
M.M. 1A	"	4300	43	5	6	0.44	4.65	2.0
M.M. 1B	"	4300	43	5	6	0.29	3.05	0.88
O.M. 36	"	2440	28	0.05	14	0.43	17.35	7.5
O.M. 24A	"	3440	36	0.05	14	0.45	15.3	6.9
O.M. 24B	"	3440	36	0.05	14		Minute	
O.M. 148A	"	2560	32	0.1	14	1.0	42.9	42.9
O.M. 148B	"	2560	32	0.1	14	0.97	39.4	38.2
O.M. 124	"	2840	30	0.1	14	1.18	17.1	20.2
O.M. 79A	"	2900	30	0.1	15	0.9	18.3	16.5
O.M. 79B	"	2900	30	0.1	15	1.55	66.5	103.1
M.M. 26	"	2500	30	1	16	0.5	17.0	8.5
O.M. 153	"	2400	19	0.1	22	0.39	2.7	1.05
O.M. 16A	"	2160	18	0.2	28	0.76	14.75	11.2
O.M. 16B	"	2160	18	0.2	28	0.80	11.7	9.4
O.M. 16C	"	2160	18	0.2	28	0.81	7.6	6.2
M.M. 18A	"	4800	48	0.2	28	1.0	49.2	49.2
M.M. 18B	"	4800	48	0.2	28	1.0	46.5	46.5
M.M. 19A	"	4800	48	1	28	0.55	14.5	8.0
M.M. 19B	"	4800	48	1	28	0.80	47.4	37.9
O.M. 151A	"	2500	22	0.1	37	0.76	11.7	8.9
O.M. 101A	"	2900	30	0.1	62	0.8	12.7	10.2
O.M. 101B	"	2900	30	0.1	62	1.32	43.9	57.9
O.M. 34	<i>Macaca trus</i>	1180	36	0.1	14	0.8	26.2	21.0
O.M. 114A	<i>Mandrillus leucophaeus</i>	3450	24	0.2	25	2.58	75.0	193.5
O.M. 114B	"	3450	24	0.2	25	2.0	78.7	157.4
O.M. 82A	<i>Presbytis entellus</i>	4060	26	0.2	39	1.28	7.1	9.1

The histological character of the oestrone-stimulated hydatids is different from the normal. In general they give considerable evidence of activity and growth, mitotic figures being frequently observed, both in the epithelium and in the stroma. The epithelial cells are much taller than normal, and they are occasionally heaped. In the normal specimens the nuclei of the epithelial cells fill the greater part of the cell

bodies (figs. 17 and 18, Plate 19). In the oestrone-stimulated specimens their size becomes relatively reduced as the cytoplasm increases in amount, and they are frequently displaced from their normal basal positions (figs. 20 and 21, Plate 19). The ciliated character of the cells is often evident (fig. 19, Plate 19), and the cells are usually secreting. This description applies not only to the surface epithelium itself, and its glandular invaginations, but also to the epithelium lining the central ducts. The surface invaginations appear more numerous in the oestrone-stimulated than in normal specimens.

The stroma of the hydatids that have reacted to the injection of oestrone is much more densely packed than is normal, and its greater density gives an appearance of relative avascularity. Frequently, too, the stromal nuclei are swollen, and in some specimens zones of sub-epithelial oedema were encountered. Extracellular granules of blood pigment were also seen in some specimens.

Unless an increase in the number of surface glandular invaginations be regarded as definite evidence of increased organization, it is difficult to decide whether oestrone promotes the differentiation as well as the growth of the hydatids of Morgagni. Only three of the fourteen normal hydatids which were histologically studied contained central ducts. Such a duct was present in eleven of the twenty-five animals that had been injected with oestrone. Moreover the epithelium of the central ducts as a rule appears more active in the oestrone-stimulated hydatids than in either the normal or the other experimental groups, even though in general the ducts do not appear to be better differentiated structures. Although these various differences may have been due to chance, there does seem to be some indication that oestrone is able to promote the differentiation as well as the growth of structures derived from the Müllerian duct.

(e) *The Reversibility of the Effects of Oestrone*

Three hydatids were recovered from two animals, O.M. 43 and O.M. 44, that were autopsied 11 and 20 days respectively after the end of 14-day courses of daily oestrone injections. Their histological appearance gives little evidence of the effects of oestrone-stimulation, and in general they resemble the hydatids recovered from normal animals.

(f) *The Inhibition of the Effects of Oestrone on the Hydatid*

A number of hydatids were recovered from animals which in addition to oestrone had been injected with some substance whose effects, it was hoped, would counteract those which oestrone induces in the prostate (ZUCKERMAN and PARKES 1936; ZUCKERMAN 1936*b*). Twenty-one hydatids were recovered from ten animals which had received oestrone and progesterone in different amounts, twenty-two from eleven animals which had received oestrone and some male hormone compound, eight from four animals which had received oestrone and cholesterol, and two from one animal which had received oestrone and epi-cholestanol.

The attempt to inhibit the prostatic effects of oestrone had been successful in some cases and unsuccessful in others. The degree to which they had been suppressed was roughly estimated and tabulated according to an arbitrary notational scale (see ZUCKERMAN and PARKES 1936 and ZUCKERMAN 1936*b*). In general it was found that the hydatids show the same degree of oestrogenic stimulation as do the prostates of the animals from which they were removed. In no case was there any unusual and specific effect that could be related to the combination of substances which the animal had received.

(g) *The Influence of Oestrone on the Hydatids of Monkeys
other than Macaca mulatta*

One hydatid was recovered from a common macaque (*Macaca irus*) that had been injected with oestrone for 14 days, and two from a drill (*Mandrillus leucophaeus*) that had been injected with oestrone for 25 days. The hydatids show the same effects of oestrogenic stimulation as do those from the rhesus monkey.

5—DISCUSSION

The hydatids of Morgagni are attached in man either to the upper pole of the testis or to the head of the epididymis, the testicular attachment being by far the commoner. The hydatids of monkeys, while often found on the testis, are on the other hand more frequently attached to the epididymis, and not only to its head, but also to its body or to the point of junction of body and tail. It is noteworthy, too, that the point of attachment is almost invariably the antero-lateral border of the body, or the antero-inferior border of the head. In no single case was a hydatid attached to the lateral surface itself.

The epididymal and testicular hydatids of monkeys cannot be distinguished from each other either macroscopically or microscopically. Moreover, clear evidence was obtained of the relation of both kinds of hydatid to remnants of a canal which on topographical grounds was taken to be the Müllerian duct. Doubts about the embryological identity of the testicular and epididymal hydatids do not therefore arise.

The testicular attachment of the hydatids in man is secondary, growth in the head of the epididymis displacing the cranial end of the Müllerian duct from the head of the epididymis where it is found in early embryonic life. It thus follows that there is a greater tendency for the embryonic condition of the Müllerian duct to persist in male monkeys than in males of the human species. A similar tendency is reflected in the fact that as a rule relatively more of the Müllerian duct appears to persist in the male monkey than in the human male. The carrying over of embryonic characters into post-natal and adult life is referred to in evolutionary discussion as neoteny, and, in general, man is regarded as being neotenic in relation to subhuman primates (BOLK 1926). In detail, however, the picture of man's neoteny or "foetalization" is an

illusory one (ZUCKERMAN 1936*d*), and it is thus not surprising to find that, so far as the persistence of the Müllerian duct is concerned, it is the male monkey which is neotenic with respect to man, and not man who is neotenic with respect to the monkey.

Remnants of the Müllerian duct are as a rule found more caudally on the testis and epididymis than are the hydatids themselves (which the duct sometimes traverses to open into the sac of the tunica vaginalis). In a few instances, however, the opposite condition held, and a fimbriated hydatid covered by ciliated columnar epithelium was situated distal to a blind duct embedded in what we have termed the Müllerian ridge of the epididymis (e.g. O.M. 82R, p. 160). This fact raises an interesting problem.

The hydatids found on the human testis and epididymis have always been regarded as derivatives of the extreme cranial end of the Müllerian duct, and their external covering of ciliated columnar epithelium has been easy to understand on the basis of the direct homology of the hydatids with the fimbriae of the Fallopian tube. The occurrence of such an external layer of epithelium would normally appear to be restricted in both male and female to the specific region where the Müllerian duct opens into the coelomic cavity. It would seem unlikely, however, that the point of attachment of "fimbriated" hydatids need necessarily be taken in all cases to represent the cranial limit of the primitive Müllerian duct. If it did, we should have to assume that during development the Müllerian duct had become considerably displaced from the Wolffian duct in those instances when a single fimbriated hydatid that is present is attached distal to a remnant of the Müllerian duct itself. In the case of O.M. 82R, for example, it would be necessary to assume first, that the cranial end of the Müllerian duct had become displaced, and second, that the duct had then doubled back on its original course.

This, however, is not the only possibility that suggests itself. It is well known that more than one fimbriated opening may occur on the same Fallopian tube, and that these accessory sets of fimbriae are sometimes found as far as 3 cm. medial to the normal opening. RICHARD (1851), who was the first to describe the condition, found such accessory openings in five out of thirty women. In his description of the development of the Fallopian tube, FELIX (1912) refers to the presence of two to four accessory "funnels" that bud in the neighbourhood of the principal funnel of the tube, with which they later fuse, the fimbriae of the ostium abdominale developing from the scattered dentations of their outer margins. He also writes of accessory "tubes", at most four in number, that occasionally appear more caudally, and that do not unite with the principal funnel. The hydatid of Morgagni which is sometimes found in the female near the ostium abdominale is said to develop from these accessory tubes, and presumably accessory openings of the tube (which are not discussed by FELIX) do the same. In the circumstances it is not unlikely that caudally disposed hydatids of Morgagni in the male monkey are derived from accessory tubes. Such a view is not supported, however, by FELIX's further observation that accessory funnels and accessory tubes do not occur in the male human embryo. On the other hand, FELIX

also maintains that the male Müllerian tubes do not develop fimbriae, a statement which it is difficult to accept in view of the occasional fimbriated character of the appendages attached to the human testis and epididymis.

The endocrinological conditions under which testicular and epididymal vestiges of the Müllerian duct persist in the male, and the sensitivity of these structures to sex hormones, are of considerable interest from the point of view of the wider problem of sex differentiation. WITSCHI (1932) has recently given an authoritative account of the investigations which have dealt with the latter question. It would appear that male and female accessory reproductive organs develop simultaneously, and grow at the same pace, during a "self-differentiating period" of embryonic life. This period ends when the gonads, by way of the "male" or "female" hormone they elaborate, assume control of the growth of the accessory organs. The duration of the self-differentiating period varies from species to species, and from organ to organ, but the effects of this period make it clear, according to WITSCHI, that "the secondary sex characters are not entirely under the control of hormones". During the "sex-controlled period" of differentiation, male or female hormones, elaborated by the developing gonads, stimulate the development of the Wolffian and Müllerian systems respectively, and the particular endocrine environment induced by the gonads leads, in some way as yet unknown, to the disappearance of the primordial reproductive system that is not being stimulated. Observations on free-martins, as well as the results of experimental parabiosis in Amphibia, show that male hormone is more dominant than female hormone, and that when a zygotic female embryo is exposed to the influence of male hormone, its Wolffian system develops at the expense of its Müllerian system, which ceases to differentiate.

The precise nature of the "male" and "female" hormones concerned in sexual differentiation has not, to our knowledge, been demonstrated. Such male hormones as were used on the animals we studied were apparently without effect on the hydatids of Morgagni, whereas they caused profound changes in organs derived from the Wolffian duct. This result is in keeping with present knowledge of embryonic development, and with the fact that none of the male hormones we used has any marked oestrogenic activity. Furthermore, the refractoriness of the hydatids to male hormone suggests that such differentiation as some of these appendages display in post-uterine life (e.g. O.M. 18) must have been effected, independently of endocrine influence, during the sexually indifferent phase of embryonic life. For, as is widely believed, male hormone is normally produced relatively earlier by genotypic male embryos than female hormone is by genotypic female embryos, and once the endocrine environment has become masculine in character the Müllerian ducts begin to retrogress, and no further differentiation is possible.

WIESNER (1935) has suggested an alternative theory of sexual differentiation which is at variance with the one expounded by WITSCHI. According to him, the Müllerian system differentiates completely in virtue of its genic constitution alone, and

independently of any endocrine influence. On the other hand, male differentiation is controlled by secretions of the testes. When "male hormone" acts on a developing female organism, it suppresses the further development of the Müllerian ducts, and promotes that of the Wolffian system. "Female hormone", having no action on the undifferentiated reproductive organs of a developing female organism, has no power to alter the course of development of the male accessory organs.

Such differentiation of the Mullerian ducts as the male monkey may show is as readily understandable on the basis of this hypothesis as it is on the basis of that advanced by WIRSCHI. A corollary of WIESNER's theory, which at the same time is one of its central supports, is the belief that oestrin cannot act on imperfectly differentiated structures derived from the Müllerian ducts. This belief is, however, contrary to the facts presented in this paper,* unless one assumes that the hydatids of Morgagni are fully differentiated structures.

Oestrone stimulates both fibro-muscular and epithelial growth in the hydatids. The response of the fibro-muscular tissue can be regarded as part of a general sensitivity to oestrogenic substances of mesodermal structures derived from the urogenital ridge and genital cord. Such a view makes it possible to relate the fact that the fibro-muscular tissue of apparently the entire genital tract is responsive to oestrone (ZUCKERMAN 1936c). Similarly, the proliferation of the epithelium of the hydatid can be related to the growth which occurs under similar conditions in the uterus masculinus. This response was first demonstrated in monkeys (PARKES and ZUCKERMAN 1935; COURRIER and GROS 1935; VAN WAGENEN 1935), but it has since been shown to occur in the dog (DE JONGH and KOK 1935), and in the guinea-pig (VAN DER WOERD 1936; COURRIER and COHEN-SOLAL 1936). In general it may be said that both the cranial and caudal parts of the primordial female reproductive tract which survive in the male mammal react to oestrone. A similar response has been shown to occur in the fowl; thus JUHN and GUSTAVSON (1932) found that the occasional vestigial remains of the Müllerian duct in the male bird also respond to oestrin. The oestrogenic sensitivity of the uterus masculinus and the hydatids of Morgagni may perhaps also be related to the occasional presence in mice which have been treated for prolonged periods with oestrone (BURROWS 1935) of keratinized cysts that lie both dorsal to the prostatic urethra and in relation to the epididymis. In this instance, however, the correspondence is not entirely clear, for the anatomical and embryological significance of the cysts observed by BURROWS is unknown; there is only presumptive evidence that they too are derivatives of the primordial female reproductive tract.

The fact that none of the Müllerian vestiges found in the male reacts to progesterone alone is of interest in view of previous observations which show that this hormone has

* The epithelial stratification observed by WIESNER in the proximal part of the urogenital sinus of the new-born rat would itself appear to be the result of oestrogenic action, and a fact at variance with his hypothesis. In the development of his hypothesis WIESNER does not consider the fact that oestrin may cause extensive changes in the male reproductive organs.

no effect on tissues that have not been previously sensitized by oestrone. The ineffectivity of progesterone is also of interest in so far as it indicates that this hormone cannot be concerned in the embryonic differentiation of the female reproductive tract. Whether or not the hormone that is concerned is an oestrogenic substance is yet to be determined. It is difficult to believe that it can be in view of the fact that these substances are not sex specific in their action.

As has been implied above, the monhormonic hypothesis of sexual differentiation would not conflict with the fact of the reactivity of the uterus masculinus and of the hydatids of Morgagni to oestrone if it were assumed that these regions of the primordial female reproductive tract became fully differentiated and determined during the sexually indifferent phase of embryonic life. The strength of such an assumption would, however, necessarily depend on the strength of this particular hypothesis, into whose experimental basis it is not necessary to enquire here. But neither on this view nor on the more conservative dihormonic hypothesis is it understandable why the cranial and caudal parts of the Müllerian ducts should continue to survive in the males of so many species after the disappearance of the rest of the ducts. These regions of the ducts, it is stated, persist in vertebrates as widely different as selachians and primates. This particular problem, however, like that of the occasional simultaneous presence in mammals of normal-sized male and female internal reproductive organs, is open only to speculation, and it is unlikely to be answered until much more is known than at present both about sex-hormone antagonisms and about the endocrine basis of sexual differentiation.

We wish to record our best thanks to Dr. A. S. PARKES, F.R.S., for his interest and help. We also wish to express our thanks to Professor W. E. LE GROS CLARK, F.R.S., for providing the general facilities which allowed this study to be carried out. The hydatids were recovered from animals which were purchased and kept with the aid of a grant to S. Z. from the Medical Research Council.

6—SUMMARY

1—Testicular and epididymal appendages of the kind described as hydatids of Morgagni were found in the following species of subhuman primate:

<i>Presbytis entellus</i> , Hanuman langur	1
<i>Cercopithecus aethiops sabaeus</i> , green monkey	1
<i>Macaca mulatta</i> , rhesus monkey	82
<i>Macaca irus</i> , common macaque	1
<i>Macaca nemestrina</i> , pig-tailed macaque	2
<i>Mandrillus sphinx</i> , mandrill	1
<i>Mandrillus leucophaeus</i> , drill	1
<i>Papio papio</i> , Guinea baboon	1
<i>Hapale jacchus</i> , common marmoset	1

The figures refer to the number of animals examined; 108 hydatids were serially sectioned.

2—No more than two hydatids were found on any single testis. As a rule only one is present, and occasionally none can be found. The epididymal and testicular hydatids are identical in structure, and both varieties may be either sessile or pedunculated, the pedicles varying greatly in size. Both "types" of hydatid are presumably derived from the Müllerian duct.

3—Epididymal are more common than testicular appendages. Their most frequent points of attachment are (a) the junction of the body and the head of the epididymis, and (b) the junction of the body and tail.

The antero-lateral border of the body is frequently raised in the form of a ridge which appears to represent the obliterated Müllerian duct. Testicular hydatids are always attached to the upper pole of the testis.

4—Remnants of the Müllerian duct are frequently found (a) within the hydatids, which they may traverse to open into the sac of the tunica vaginalis, (b) in the pedicle of the hydatids, and (c) in the "Müllerian" ridge on the antero-lateral border of the epididymis. Occasionally a hydatid and its duct are so well differentiated that they resemble a miniature Fallopian tube.

5—In one specimen the remains of the Müllerian duct communicated with both the sac of the tunica vaginalis and a cyst formed by the expansion of a vas aberrans of the head of the epididymis.

6—Oestrone stimulates growth in both the epithelial and stromal elements of the hydatids; the evidence was insufficient to decide whether or not it also promotes their differentiation. No definite effect on the hydatids could be noticed after treatment with either a gonadotropic extract of the anterior lobe of the pituitary, or progesterone, or male hormone.

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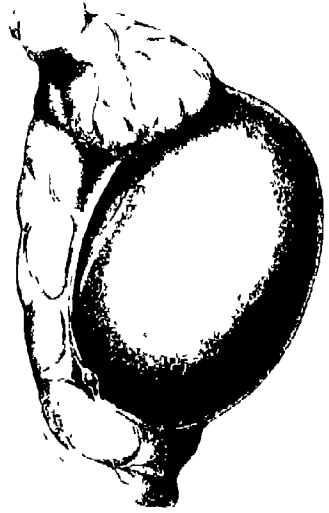
DESCRIPTION OF PLATES

PLATE 15

FIG. 1—Hydatids of Morgagni in normal and experimental monkeys. $\times 3$. 148R, after oestrone injections; 151R, after oestrone injections; 163R, normal; 179R, after oestrone and male hormone injections; 170R, after oestrone and male hormone injections; 82R, after oestrone injections.



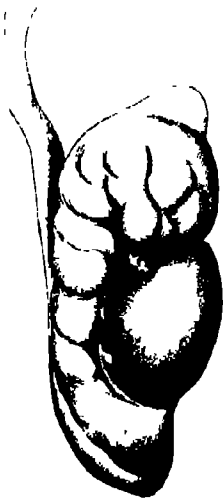
148 R



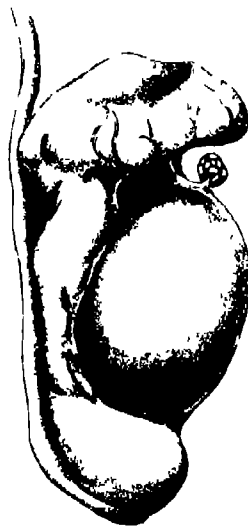
163 R



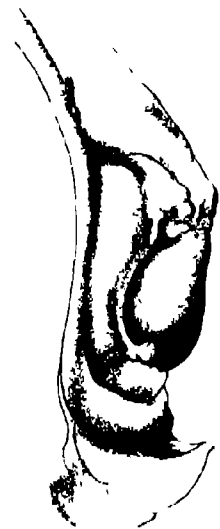
151 R



179 R



170 R



82 R

PLATE 16

Remains of the Müllerian duct in the male monkey

FIG. 2—O.M. 160. A hydatid of Morgagni attached to the superior epididymal ligament, which contains a remnant of the Müllerian duct. $\times 29$.

FIG. 3—O.M. 160. A more caudally disposed second remnant of the same Müllerian duct. There is no sinus epididymis. $\times 29$.

FIG. 4—O.M. 163R. A cross-section of the vesicle shown on the "Müllerian" ridge of the epididymis in fig. 1, 163R. The vesicle represents part of the Müllerian duct. $\times 56$.

FIG. 5—O.M. 163L. A hydatid of Morgagni deeply embedded between the upper pole of the testis and the head of the epididymis. $\times 29$.

FIG. 6—O.M. 82R. Cross-section of the hydatid 82R, fig. 1, at the junction of the body and tail of the epididymis. A central duct in the hydatid, remains of the Müllerian duct, is shown opening into the sac of the tunica vaginalis. $\times 56$.

FIG. 7—O.M. 82R. Remains of the Müllerian duct in the "Müllerian" ridge of the epididymis, cranial to the attachment of the hydatid shown in fig. 6. $\times 56$.

FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIG. 7



PLATE 17

The normal hydatid of Morgagni in different species of monkey

FIG. 8—O.M. 90. Immature rhesus monkey, *Macaca mulatta*. Hydatid of Morgagni of Type III. Note the sparse stroma, and the line of junction of the columnar epithelium of the hydatid, with the flat serous epithelium of the pedicle. $\times 78$.

FIG. 9—M.M. 22. Similar to fig. 8, but from a Guinea baboon, *Papio papio*. $\times 50$.

FIG. 10—O.M. 20. Hydatid of Type III from a mature rhesus monkey, *M. mulatta*. $\times 40$.

FIG. 11—O.M. 35. Hydatid of Type IV from a green monkey, *Cercopithecus aethiops sabaeus*. The hydatid is attached to the testis and has a central duct that opens into the sac of the tunica vaginalis. $\times 70$.

FIG. 12—O.M. 144. Hydatid of Type III from a rhesus monkey that had been injected with oestrone. Note the large number of tubular invaginations of the surface columnar epithelium. The folds they produce are to be distinguished from the more prominent ones shown in fig. 10, and which give the hydatid a fimbriated appearance. Note also the fact that the stroma is more densely packed than it is in normal specimens. $\times 75$.



FIG. 8



FIG. 9



FIG. 10



FIG. 11



FIG. 12

PLATE 18

Remains of Müllerian duct found in the hydatids themselves: rhesus monkeys

FIG. 13—M.M. 18. Large blind cyst in pedicle of hydatid. The animal had been injected with oestrone. $\times 20$.

FIG. 14—M.M. 18. Hydatid on opposite testis of same monkey, showing a large, coiled and folded central duct which opens into the sac of the tunica vaginalis. $\times 29$.

FIG. 15—O.M. 16. A blind central duct within a hydatid itself. $\times 50$.

FIG. 16—O.M. 114. The opening of a central duct into the sac of the tunica vaginalis. The distinction between the central duct and the tubular invagination of the surface epithelium on the right side can be readily made out. The line of junction between the columnar epithelium of the hydatid and the flat serous epithelium of the pedicle is clearly shown. $\times 50$.

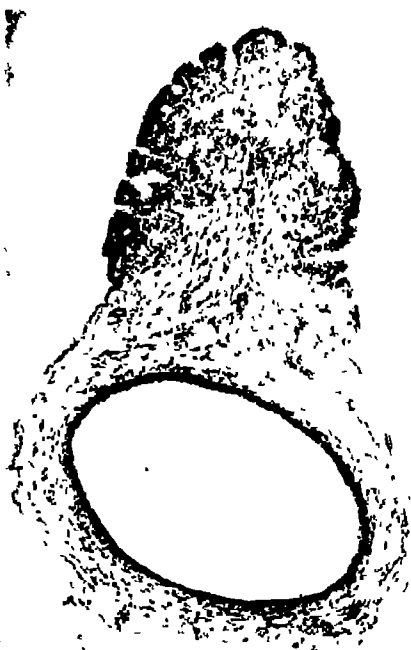


FIG. 13



FIG. 14

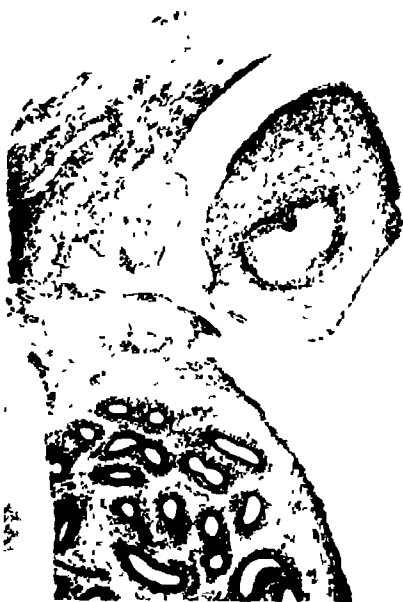


FIG. 15



FIG. 16

PLATE 19

Normal and oestrone-stimulated epithelium of hydatids. $\times 600$.

FIG. 17—P. 6. Surface epithelium of hydatid of normal pig-tailed macaque, *Macaca nemestrina*. The nuclei are relatively large. The dispersion and fibrillar nature of the stromal elements is also shown.

FIG. 18—O.M. 90. Rhesus monkey. Same as fig. 17.

FIG. 19—O.M. 101. Rhesus monkey, after oestrone injections. The ciliated character of the cells lining the numerous tubular invaginations of the surface epithelium can be seen.

FIG. 20—O.M. 79. Rhesus monkey. The figure shows the tall surface epithelium following oestrone injections. The nuclei are no longer basally disposed. Two of the cells are in process of division.

FIG. 21—O.M. 16. The tall columnar epithelium of the central duct of a hydatid of a rhesus monkey following oestrone injections, showing a mitotic figure.

FIG. 17



FIG. 18

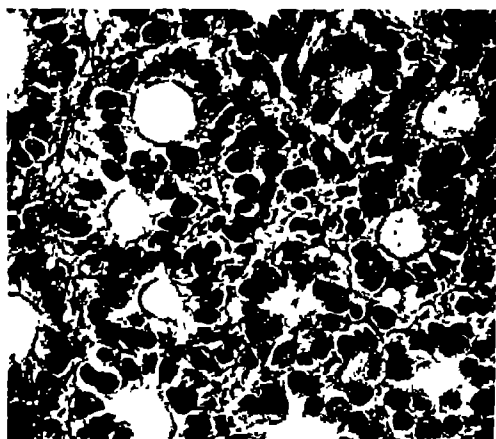


FIG. 19



FIG. 20

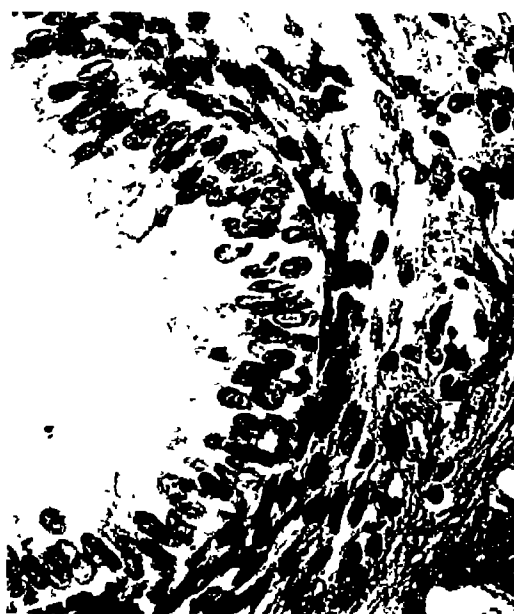


FIG. 21

PLATE 20

The intercommunication of Müllerian and Wolffian structures. O.M. 135. $\times 22$.

FIG. 22—The lowermost epididymal lobule seen in the figure is a blind coiled duct which expands to form a cyst.

FIG. 23—The cyst is shown in this figure, and closely applied to it is a hydatid of Morgagni. The deepest tubular gland in the hydatid is a central duct, presumably the cranial end of the Müllerian duct, which connects the cavity of the cyst with that of the tunica vaginalis.

FIG. 24—The central duct is seen in the centre of the hydatid.

FIG. 25—This figure shows the central Müllerian duct opening into the epididymal cyst, to which the hydatid is attached.

FIG. 26—The epithelium of the distal part of the cyst is folded; the figure shows one of the two accessory cysts which are situated in this region.



FIG. 22



FIG. 23



FIG. 24



FIG. 25



FIG. 26

IV—ON THE MORPHOLOGY OF THE ALIMENTARY CANAL, PROCESS OF FEEDING, AND PHYSIOLOGY OF DIGESTION OF THE NUDIBRANCH MOLLUSC *JORUNNA TOMENTOSA* (CUVIER)

By NORMAN MILLOTT, M.Sc., *Demonstrator in Zoology in the
Victoria University, Manchester*

(Communicated by H. Graham Cannon, F.R.S.—Received 27 January, Read 29 April 1937)

I—INTRODUCTION

Jorunna tomentosa is a nudibranch which feeds on a diet of sponges.

It was thought that an investigation of the anatomy and physiological processes of the gut of such an animal might yield interesting results, by comparison with those gained by investigators of less specialized molluscs. The following account aims at providing a detailed description of the anatomy and histology of the gut, of the mechanism of feeding, and physiology of digestion of *Jorunna tomentosa* which are compared, so far as possible, with similar structures and processes in other molluscs and phyla.

It is with great pleasure that I acknowledge the invaluable help of Professor H. GRAHAM CANNON, F.R.S. I also wish to thank Dr. C. F. A. PANTIN for much valuable help and advice. My thanks are also due to Professor L. E. S. EASTHAM for his continued interest and advice, and to Professor E. A. SPAUL for permission to use the Leeds University Laboratory at Robin Hood's Bay, where a portion of the work was carried out.

II—METHODS

The anatomy was determined as far as possible by dissection, aided where necessary by reconstruction from serial sections in the transverse, longitudinal and frontal planes.

Various staining and fixing methods were employed for the investigation of the histology. The statements made by other workers concerning the results given by fixatives are confusing. NICOL (1930), working on the gut of *Sabella*, found that Duboscq-Brasil gave excellent results, while chrome-osmic mixtures such as Flemming (with and without acetic) and Champy proved unsatisfactory. GRAHAM (1932), working on the gut of *Patella*, found that exactly the opposite was the case. Both Flemming without acetic (alone, and in the form of Champy-Kull's modification) and Duboscq-Brasil were accordingly used, both proving useful in demonstrating various structures within the cell. It was found that Flemming without acetic proved

most satisfactory in demonstrating cilia, cell inclusions, and nuclear detail, and in differentiating various kinds of cytoplasm, while Duboscq-Brasil proved most satisfactory in demonstrating the presence of intracellular fibrils. Neither GRAHAM's contention that fixation in Duboscq-Brasil refused to differentiate between the various epithelia of the gut, nor NICOL's statement that Flemming without acetic gave unsatisfactory results, were borne out by this investigation.

In addition, Bouin's aqueous fluid, corrosive acetic, and Susa fixatives were used; the first proved relatively valueless, the second useless for demonstrating cell inclusions, but useful in combination with Mallory's triple stain for picking out connective tissue, while the latter proved excellent for fixing cilia, basal granules, and intracellular fibrillae.

After Flemming without acetic, safranin and light green and the Champy-Kull stain (BOLLES LEE, p. 333) were employed to demonstrate nuclear and cytoplasmic detail. For general reconstruction, Delafield's haematoxylin and eosin, Mann's methyl-blue eosin, and Mallory's triple stain were used. Meyer's mucicarmine with orange G as counterstain was used for the demonstration of mucus cells.

III—BIONOMICS

Jorunna is commonly found round our coasts, crawling on the under-side of large slabs of rock at low-water mark. Here it is found rasping away such sponges as *Halichondria*, making visible trails as it passes inwards into the sponge mass.

IV—MORPHOLOGY OF THE ALIMENTARY CANAL

1—General Anatomy (fig. 1)

The mouth leads into a buccal cavity (*b.m.*) with very muscular walls and provided with a triturating apparatus. The buccal cavity passes into a simple oesophagus (*oes.*), which runs backwards to open into a sac-like region (*M.G.*) situated roughly in the middle of the animal and receiving the openings of a complex racemose gland, the so-called "digestive gland" or "liver". Dorsally the sac-like region narrows to form the tubular intestine (*int.*), which, running forwards to the anterior end of the body, bends round upon itself and passes backwards to the mid-dorsal anus (*a.*).

For purposes of description the gut may be divided into foregut, midgut, and hindgut.

2—Detailed Anatomy

The Foregut (fig. 2)—The buccal mass has an irregular shape, protruding anteriorly at the mouth, while posteriorly it is produced into the radula sac ventrally, and the oesophagus dorsally (figs. 1 and 2, *r.s.* and *oes.*). The walls are thick and muscular, and in addition the whole mass can be rotated in a sagittal plane about the mouth, by the action of extrinsic muscles. The buccal mass is figured and described in the position

of retraction, that is, when the radula sac is pressed against the floor of the body cavity.

The probosciform mouth (*m.* fig. 2) is in the form of a hollow cylinder projecting forward from the buccal mass and surrounding an inner cylinder, also forwardly projecting, consisting of three so-called "lips" (ALDER and HANCOCK 1845), which are freely movable and can be protruded through the mouth, as shown in fig. 2. Commencing at the anterior end of this inner tube, the first or outer lips (*o.l.* fig. 2) form a short plicated tube running posteriorly to end in a muscular ring-like thickening, the

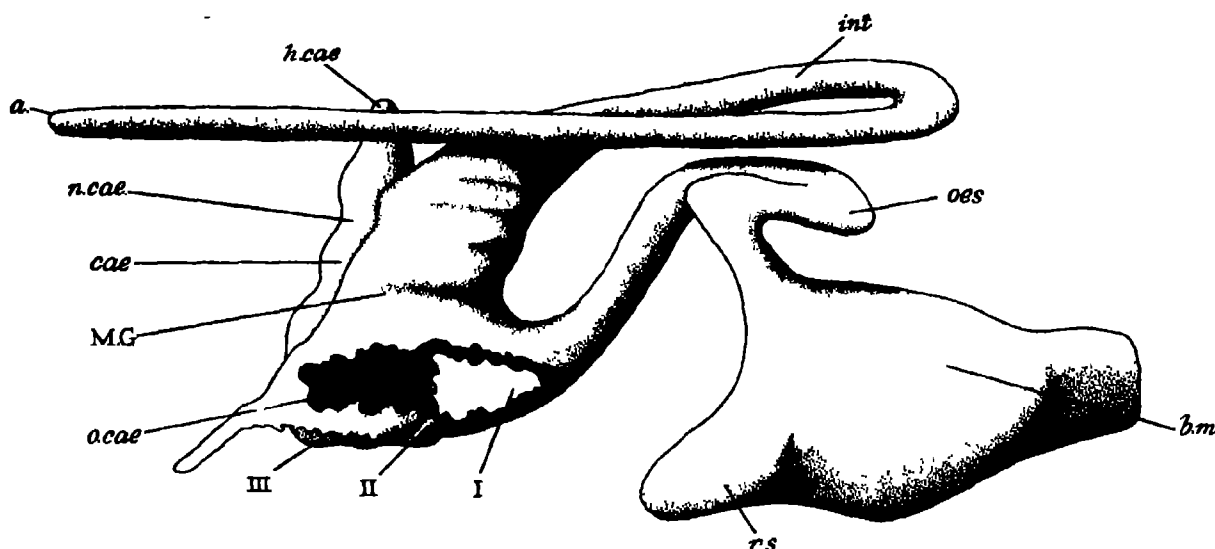


FIG. 1—A reconstruction of the gut viewed from the right side, so as to display the midgut; the digestive gland, which normally surrounds it, has been omitted. *N.B.* This reconstruction was not drawn with numerical accuracy. An endeavour has been made to obtain the correct proportions by correlating transverse, longitudinal and frontal sections. *a.* anus; *b.m.* buccal mass; *cae.* caecum; *h.cae.* head of caecum; *n.cae.* neck of caecum; *int.* intestine; *M.G.* midgut; *o.cae.* opening of caecum into midgut; *oes.* oesophagus; *r.s.* radula sac; *I, II* and *III*, openings into digestive gland. \times about 15.

"inner lips" (*i.l.* fig. 2), which in turn are succeeded almost immediately by another ring-like thickening, the extremely large and muscular "buccal lips" (*b.l.*). The lips can be protruded through the mouth opening (see "Feeding", § VI).

Behind the buccal lips lies the main cavity of the buccal mass which houses the odontophore (*od.*) with its radula (*rad.*). From the dorsal aspect of this cavity arises the oesophagus, from the ventral aspect the radula sac (*r.s.*).

The odontophore is a muscular tongue-like prominence arising from the floor of the buccal cavity. Its surface, remote from the buccal lip, is deeply grooved down the middle. Applied to this surface, and passing into the groove, is the chitinous ribbon or radula, beset with transverse rows of recurved teeth. The radula thus appears

bilobed: in fig. 2, which is a sagittal section of the buccal mass, one lobe of the radula is seen in surface view. The form of the radula and the number and arrangement of teeth have been described by ALDER and HANCOCK (1845). Only a portion of the radula is exposed, the remainder is housed in the radula sac which forms a conical

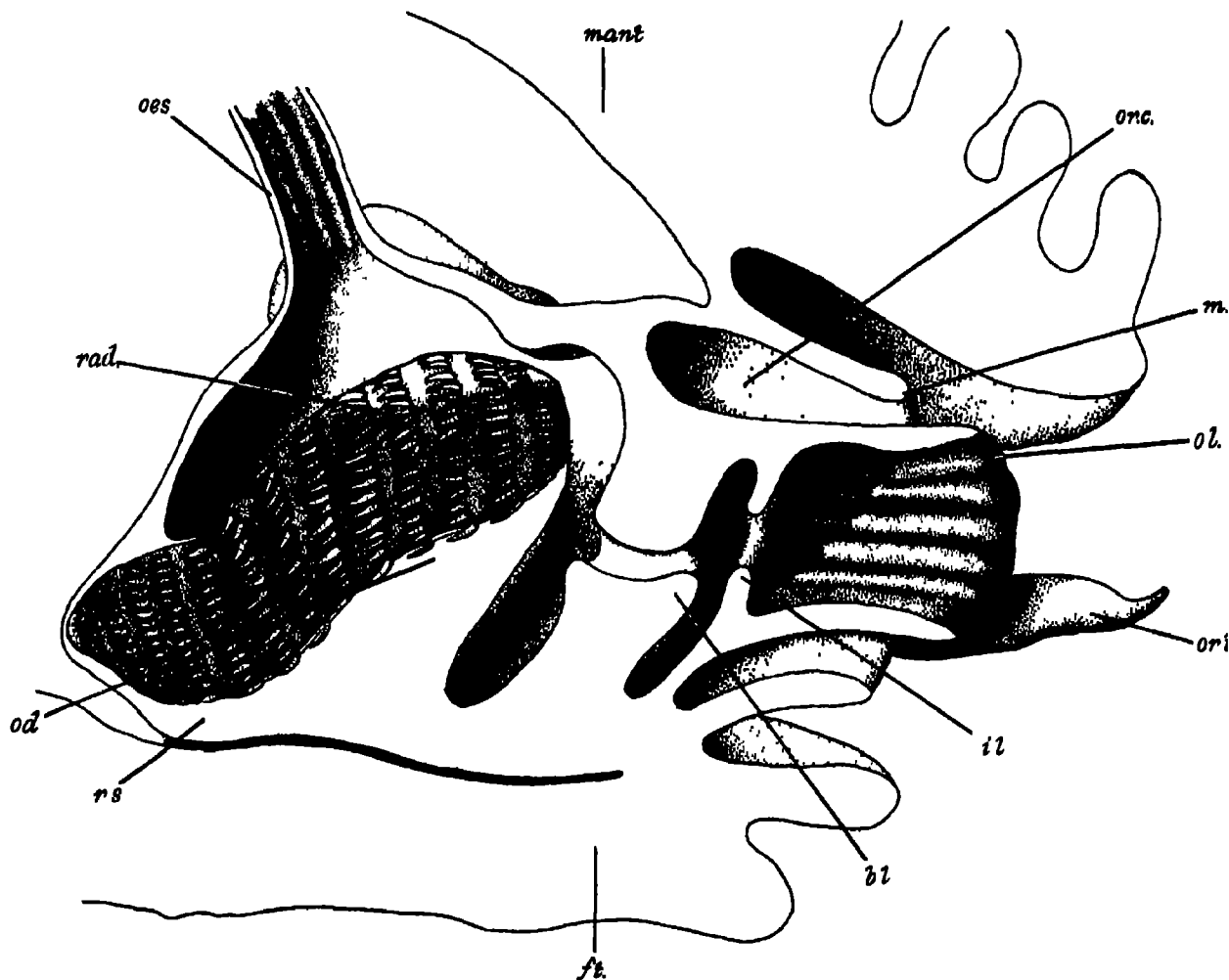


FIG. 2.—Sagittal section of the head region of *Jorunna* showing the buccal mass and associated structures. Cut surfaces are left blank. *N.B.* No attempt has been made to show the precise number, and arrangement, of the teeth of the radula. *b.l.* buccal lip; *ft.* foot; *i.l.* inner lip; *m.* mouth; *mant.* mantle; *od.* odontophore; *oes.* oesophagus; *o.l.* outer lip; *or.t.* oral tentacle; *or.c.* oral channel; *rad.* radula; *r.s.* radula sac. \times about 30.

projection from the postero-ventral angle of the buccal mass. Partly as a protection against the radula teeth themselves, and partly to provide an effective surface against which the teeth can crush and macerate the food, the walls of the buccal mass, from the inner lips inwards, are covered with a thick cuticle.

The term "oesophagus" is applied to the rest of the foregut by ALDER and HANCOCK. The term is widely used, and is retained here; but it is unfortunate, since it implies a

function comparable with that of the oesophagus of vertebrates, whereas the oesophagus of *Jorunna* has a different physiological significance.

The actual course of the oesophagus varies according to the movements of the buccal mass. Thus when the buccal mass is erected, the oesophagus takes the form of a straight tube, as figured by ALDER and HANCOCK, but when retracted, that is, pulled down, it takes a more or less tortuous course as in fig. 1. The exact course has little significance in this account, and it is best regarded as a straight tube running backwards to open into the sac-like midgut.

Internally the lining of the oesophagus is thrown into pliable longitudinal muscular ridges, the shape of which, and their relations to one another, as well as their relative sizes, varies enormously with the state of distension of the gut with food, and with the tonus of the muscles of the gut wall. Though the ridges may be obliterated in varying numbers and to a varying extent, they return to their original shape when the pressure is removed; usually, examination of the inner surface of the wall of the oesophagus in the living animal reveals the presence of nine distinct longitudinal ridges.

Each of these "primary" ridges is thrown into a series of secondary ridges which run at right angles to the direction of the former, and meeting their fellows from adjacent ridges, form a series of hoops around the lumen of the foregut. These secondary folds occur throughout the oesophagus and, like the primary folds, they are pliable and even more easily obliterated by the gut contents, but return to their original form when the pressure is removed.

The secondary ridges contrast with the primary ridges, first, in being only slight elevations of the surface whereas the primary ridges may be so raised up as almost to block the lumen of the gut, and secondly, in having no significance as food grooves (see p. 201).

Salivary Glands—Neither careful dissection, nor examination of serial sections, revealed the presence of any salivary glands in connexion with the foregut. In this feature *Jorunna* differs from *Archidoris*, which possesses large salivary glands that are figured and described by ALDER and HANCOCK (1845).

The Midgut—The midgut follows the oesophagus and receives the openings of the digestive gland.

The midgut is very difficult to see by dissection, first because it is surrounded by the complex digestive gland, which must be carefully teased away before it can be reached, and secondly because its walls are exceedingly thin and easily damaged. It has therefore been found necessary to reconstruct its anatomy largely from serial sections.

The midgut may best be regarded as a very regular sac (*M.G.* fig. 1) receiving the oesophagus anteriorly and passing dorsally into the tubular hindgut (*int.* fig. 1); its walls are pierced by four openings, one leading into the caecum (*cae.*) and the other three into the surrounding digestive gland. The most anterior of these latter three

openings is ventro-lateral in position, encroaching over about half the left side (I, fig. 1). The second or lateral opening occupies the whole of the right side (II, fig. 1), while the third and posterior opening occupies practically the whole posterior aspect of the midgut (III, fig. 1). In addition a diverticulum or caecum (*cae.*) opens into the left-hand side, its opening (*o.cae.*) occupies the remaining half of this aspect.

In consequence of the four large openings, the midgut walls are very limited in extent, and in sharp contrast with the foregut and hindgut, they are attached by connective tissue to the surrounding liver mass.

The Digestive Gland—The digestive gland is a complex racemose gland formed of a mass of blind, branching tubules separated by strands of connective tissue. It is closely invested by the gonad, parts of which tend to penetrate the connective tissue between the tubules. These tubules are collected into three large channels which open into the midgut by the large openings described above.

The Caecum—The caecum or diverticulum forms a blind sac communicating with the hindgut (fig. 1).

Partly because it is a minute structure, and partly because it is deeply embedded in the tissue of the digestive gland, it proved impossible to investigate its structure by dissection. The following account of its anatomy is based entirely on reconstruction from serial sections cut in the transverse and frontal planes.

The caecum is thin-walled and exceedingly delicate, and its structure is shown in fig. 3. The expanded ventral portion or fundus (*f.*), which has a large opening (*o.cae.* figs. 1 and 3) into the left side of the midgut, passes dorsally into a narrow neck-like region (*n.cae.* figs. 1 and 3) which in turn expands to form the blind head of the caecum (*h.cae.* figs. 1 and 3).

The walls remote from the midgut are thrown into pliable muscular ridges which, like those of the oesophagus, may be temporarily obliterated or distorted by the pressure of contents. There are two series of ridges, a mesial series (*mes.r.*), running dorso-ventrally, and a lateral series on either side (*a.lat.r.* and *p.lat.r.*), running obliquely upwards and inwards from the fundus into the neck region.

The head has thicker and more muscular walls than the rest (see p. 188).

The Hindgut—The hindgut arises from the dorsal aspect of the midgut as a simple dilated tube subcircular in cross-section. It passes dorsally and forwards slightly to the left of the middle line, progressively narrowing as it does so. Passing forward to a point approximately overlying the origin of the oesophagus from the buccal mass (fig. 1), it curves towards the right and, continuing its curvature, passes over to the right-hand side of the body, along which it passes backwards as a simple uniform tube curving gradually towards the median line to reach the anus, which lies amid the gills at the posterior end in the mid-dorsal line.

The first dilated portion of the hindgut is frequently called the "stomach" (ALDER and HANCOCK) and corresponds with the similarly named greatly dilated portion in

Archidoris (ALDER and HANCOCK 1845, and YONGE 1925*b*). The remaining portion which passes back to the anus as a simple uniform tube is frequently called "intestine". For these regions in *Jorunna*, both these names are unsatisfactory, since no enzymes are produced, neither does digestion or absorption occur (as the suggested names would imply). The internal surface, from the point of emergence from the midgut to the anus, is thrown into primary and secondary ridges exactly like those of the oesophagus.

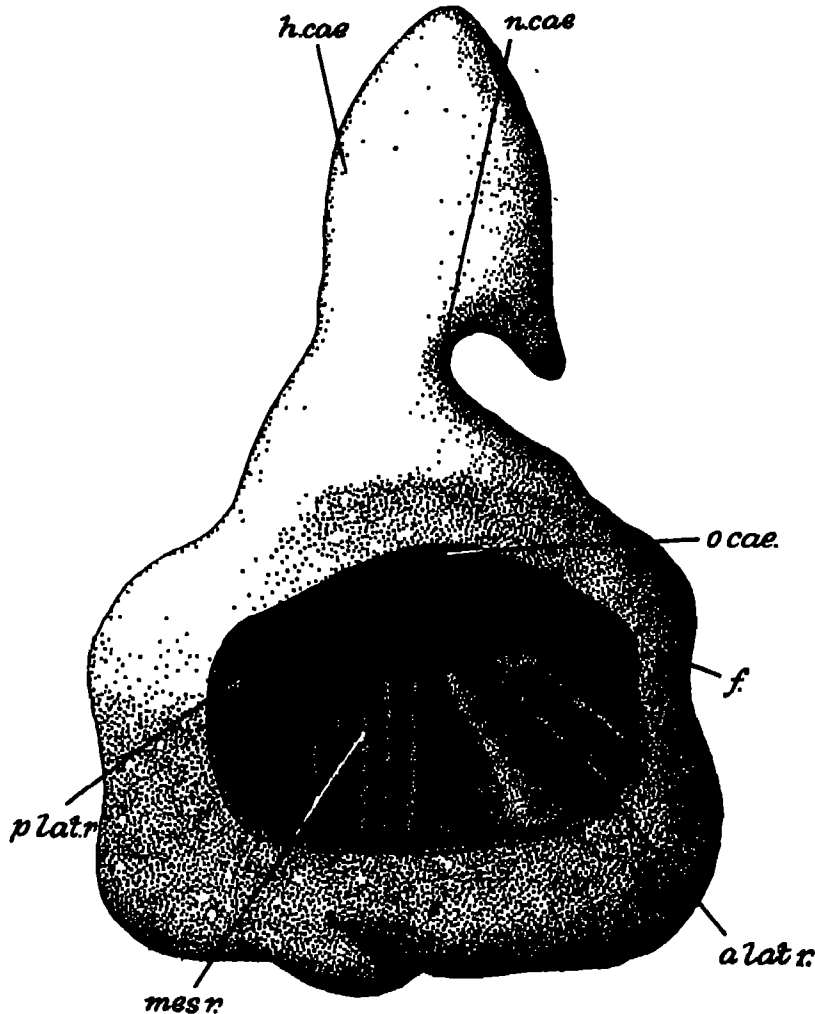


FIG. 3—Reconstruction of caecum, viewed from the right side. *a.lat.r.* anterior lateral ridge; *f.* fundus; *h.cae.* head of caecum; *mes.r.* mesial ridge; *n.cae.* neck region; *o.cae.* opening of caecum into midgut; *p.lat.r.* posterior lateral ridge. \times about 52.

V—HISTOLOGY

1—*The Buccal Mass* (for anatomy see p. 174)

In the following account the histological structure of the buccal cavity proper (i.e. the cavity beyond the inner lips) is described. No attempt is made to describe the

histological structure of the radula sac, which would involve a discussion of the formation of the radula and its teeth, a subject which is beyond the scope of this account.

Details of the formation of the radula in molluscs generally are given by SOLLAS (1907a).

Fig. 4 is a transverse section of the wall of the buccal mass as seen after fixation in Flemming without acetic and stained in safranin and light green.

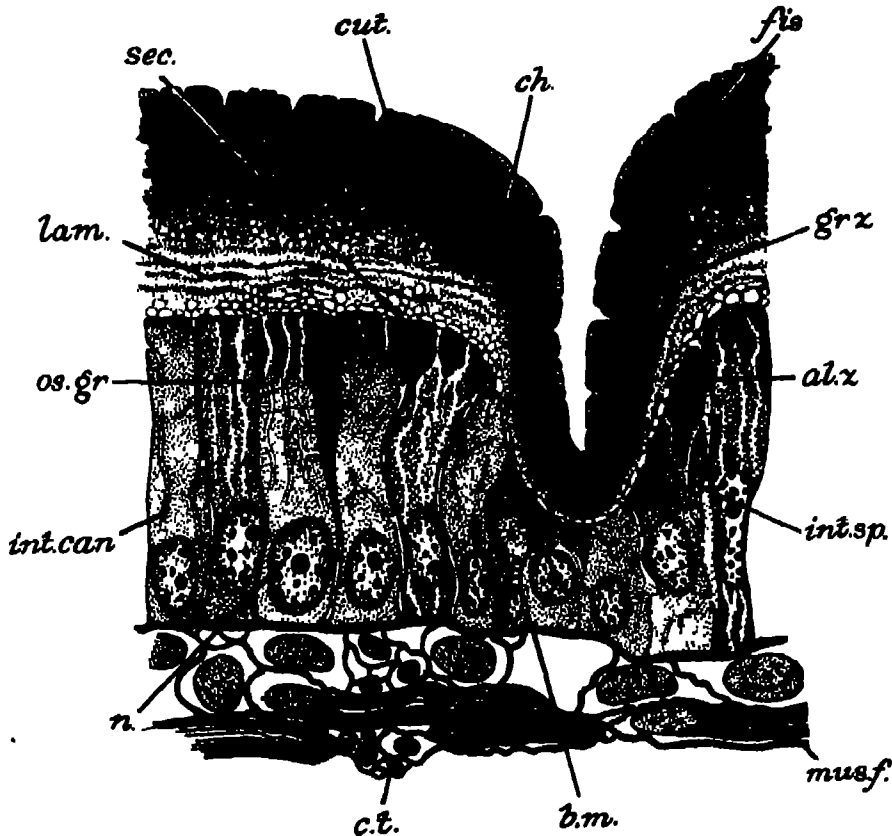


FIG. 4—Transverse section of the wall of the buccal mass. Fixed Flemming without acetic, stained safranin and light green. *al.z.* alveolar zone; *b.m.* basement membrane; *ch.* chitin; *c.t.* connective tissue fibre; *cut.* cuticle; *fis* fissure in chitin; *gr.z.* granular zone; *int.can.* intracellular canaliculus; *int.sp.* intercellular canaliculus; *lam.* lamina of laminated zone; *mus.f.* muscle fibre; *n.* nucleus; *os.gr.* granules appearing greyish after osmic acid; *sec.* apparent secretion of chitin from intercellular canaliculus. $\times 1600$.

The gut wall is conspicuous here by its possession of a cuticular layer which appears differentiated into zones. These are particularly easily distinguished where the layer is thick, as for example on the buccal lips. It is assumed that this layer is chitinous, since a similar layer found in similar situations in certain other molluscs has been shown to exhibit the same chemical and physical properties as the chitin of arthropods (SOLLAS 1907b; WESTER 1910). The zones of the chitin are as follows:

Nearest the epithelium is an alveolar zone (*al.z.*), followed by a laminated region, the laminae (*lam.*) usually staining a pale green (see below). Following this is a granular zone (*gr.z.*), the granules becoming compacted distally to merge into a broad zone of solid chitin (*ch.*), the continuity of which is broken only by vertical and longitudinal fissures. This is followed by a shallow zone (*cut.*) which is more retentive of safranin than the underlying chitin, and, as it forms the outermost layer, may be termed cuticle. The alveolar, laminated and granular zones may be absent.

The reactions of the chitin and cuticle to various stains is shown in Table I.

TABLE I

Stain	Chitin	Cuticle
Meyer's mucicarmine	Does not stain	May appear pinkish or unstained
Orange G	Yellow	Does not stain
Sauré fuchsin	Pink or reddish	Red
Safranin and light green	Green and/or red	Green and/or red
	The cuticle tends to retain safranin more readily than the chitin	
Mallory	Orange and/or blue, and/or red	Orange and/or blue, and/or red
	The zone of chitin nearest the epithelium always stains blue	
Delafield's haematoxylin	Pinkish	Pinkish
Mann's methyl-blue-eosin	Colours varying from blue to red are common to both chitin and cuticle	

The underlying chitogenous epithelium consists of one type of cell only. These are usually columnar, though they vary greatly in shape and dimensions according to the exact region in which they are observed.

Their outstanding feature is the possession of intracellular canaliculi (*int.can.*) which open either at the free surface of the cell, or into intercellular canaliculi (*int.sp.*). Within the cell the canaliculi become subdivided into numerous branches, all of which end blindly. The main course of these branches appears to be parallel to the long axis of the cell.

Usually the intracellular canaliculi and intercellular spaces appear clear, but often they appear to contain a secretion which stains a bright red in safranin, and blue in Mallory. The significance of this secretion is discussed below. Frequently the upper third of the cytoplasm either stains more deeply, or contains numbers of granules (*os.gr.*), which appear greyish after osmic fixation or may take up light green or safranin.

The nuclei (*n.*) are basal, ovoid, and possess a prominent nucleolus and scattered irregular chromatin masses.

The epithelium rests on a basement membrane (*b.m.*) which overlies the main part of the wall of the buccal mass which is thickly beset with connective tissue (*c.t.*), and muscle fibres (*mus.f.*), running in all directions.

The most significant features of the gut wall of this region are the possession of chitin and cuticle, and a very well-developed muscular layer.

The hard surface of the chitin, combined with a certain amount of flexibility, no doubt enhanced by the fissures running through it, together with the powerful

musculature, provide an effective combination for the titrating function of the buccal mass.

Fixation in Flemming without acetic demonstrates the zonation of the chitin particularly clearly. After fixation in Duboscq-Brasil and Susa's fluids, however, the chitin appears relatively homogeneous, and the zonation described above is not so clear.

It may be argued, in view of the above, that the zonation is due to bands of precipitate (Liesegang effect), being formed by the action of a particular fixative. That this is not so, is shown by the fact that the zonation always runs parallel to the free surface of the epithelium, and never parallel to the vertical fissures, or in any other plane.

The intracellular canaliculi are particularly clearly demonstrated by fixatives such as Duboscq-Brasil and Susa, which tend to dissolve the cell contents and cause shrinkage. Though the canaliculi may be seen after fixation in Flemming without acetic, their course is more difficult to follow than when fixed in the former fluids.

Discussion—VITZOU (1882) has expressed the view that the chitin of the integument of decapod Crustacea arises by gradual transformation of the outer layers of the cells of the chitogenous epithelium. Thus from each cell a minute block of chitin is produced, one row of such blocks forming a lamina of the fully formed chitin. The blocks become separated off from the cells, and since, as the process goes on, the chitinous blocks produced by adjoining cells do not fuse, the chitin eventually comes to consist of multi-layered cylinders.

A consideration of the histological features of the chitogenous epithelium of the buccal mass of *Jorunna* does not accord with this view, and seems to indicate that the chitin here does not arise by transformation of the outer layers of the epithelial cells, but that it is secreted by these cells.

The particular features affording support to this view are the following:

First, if the chitin consisted of multi-layered cylinders, we would expect to find at least slight evidences of vertical striations running through the chitin. No such striations have been observed.

Secondly, the alveolar layer presents the appearance of being a secretion, as shown particularly by the way in which it dips down into the intercellular canaliculi.

This appearance, coupled with the occasional appearance of a secretion in the intercellular and intracellular canaliculi seems, to indicate that the secretion in question is passed out through the canaliculi. The staining reactions of the secretion in the canaliculi lend support to this view, for though the chitin as a whole stains in an arbitrary fashion (see below), the newest layers of chitin (i.e. those nearest the epithelium) always stain blue in Mallory, as does the secretion in the canaliculi.

This view is in accordance with the views expressed by BLOCH (1896), SOLLAS (1907*a*), and others, concerning the origin of the radula and its teeth. They believe them to be secreted and not to arise by transformation of the formative cells.

The absence of any definite ducts through the chitin, and any associated glands,

renders it clear that the cuticle cannot arise in a manner similar to that described by YONGE (1932*b*) for the decapod Crustacea, and it must therefore arise either by the transformation of the peripheral layers of chitin into a cuticular substance, or in a manner similar to that described for *Rhodnius prolixus* by WIGGLESWORTH (1933), where he suggests that the underlying epithelium secretes the cuticle first, followed by the chitin afterwards. The former suggestion is supported by the fact that the chitin of the radula changes its properties with age. This was shown by SOLLAS (1907*a, b*), who demonstrated that the young and old portions of the radula differed in chemical and physical properties, and by PANTIN and ROGERS (1925), who found that the older portion of the radula of *Buccinum undatum*, unlike the younger portion, was amphoteric.

The reaction of chitin and cuticle to stains as shown by a study of the buccal mass of *Jorunna* is not in agreement with the results gained by YONGE in the decapod Crustacea. This affords further support to the idea that the cuticle in *Jorunna* does not arise in a manner similar to that described by YONGE for cuticle of the decapods. YONGE states that the chitin and cuticle *always* stain differently. This was not found to be so in *Jorunna*, as Table I on p. 181 shows. Moreover, Professor CANNON has informed me privately that he has not found this difference of staining reaction between chitin and cuticle to be constantly manifest in the Entomostraca, and has shown me sections of a rare arachnid in which the staining reactions were reversed.

2—The Oesophagus (for anatomy see p. 177)

In considering the remainder of the gut from the histological standpoint, it is no longer sufficient to retain the division of the gut into regions such as we have hitherto used, since both the oesophagus and hindgut can be subdivided into two regions which differ appreciably in the characters of their lining epithelium, and on the other hand two such morphologically distinct regions as the midgut and a section of the oesophagus have lining epithelia which are precisely similar.

Therefore it is proposed, while discussing the histological features of the gut, to adopt a scheme based on that used by GRAHAM (1932) in his description of the gut of *Patella*, and to subdivide the oesophagus and hindgut into sections A and B (fig. 5).

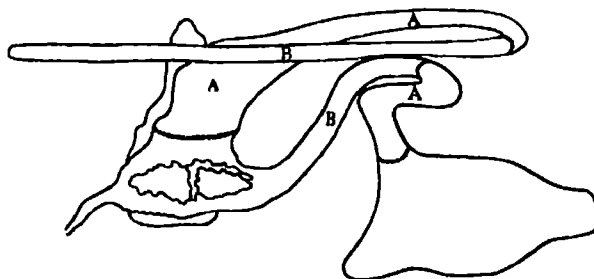


FIG. 5—Diagram showing scheme of subdivision of gut into histological regions. The broken lines indicate the approximate boundaries between the regions.

1—*The Oesophagus, Section A.* The main histological features of section A are shown in fig. 6, which is a transverse section of the gut wall of this region, as seen after fixation in Flemming without acetic and staining in safranin and light green.

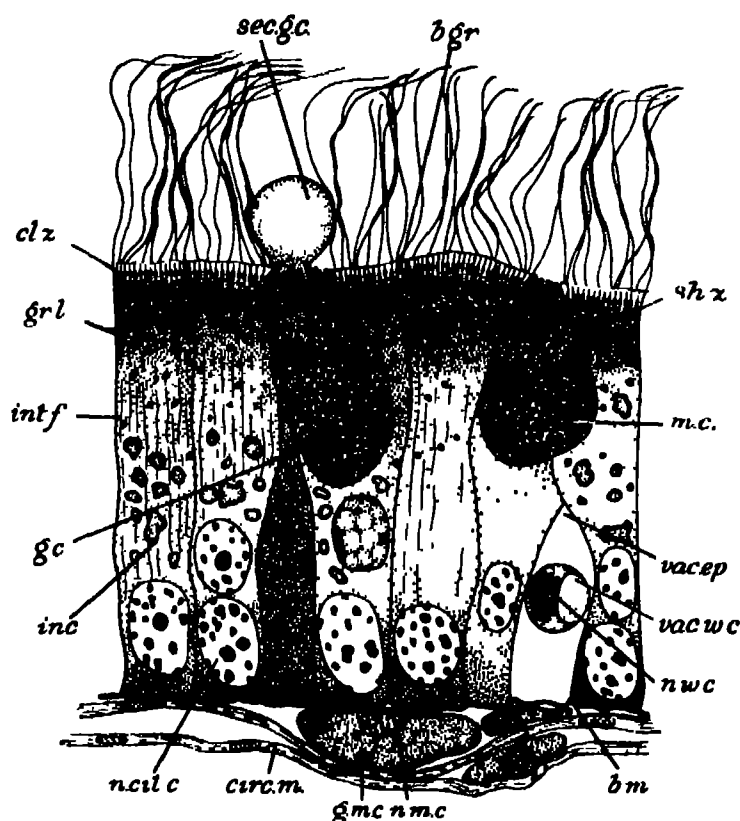


FIG. 6—Portion of epithelium of section A of the oesophagus. Fixed Flemming without acetic, stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *circ.m.* circular muscle; *cl.z.* clear zone; *g.c.* gland cell; *g.m.c.* giant mucus cell; *gr.l.* layer of irregular granules; *inc.* solid inclusions appearing greyish after osmic fixation; *int.f.* intracellular fibrils; *m.c.* mucus cell in epithelium; *n.cil.c.* nucleus of ciliated cell; *n.m.c.* nucleus of mucus cell of submucosa; *n.w.c.* nucleus of wandering cell; *sec.g.c.* secretion of gland cell; *sh.z.* zone beneath basal granules; *vac.ep.* vacuole in epithelium; *vac.w.c.* vacuole in wandering cell. $\times 1330$.

After osmic fixation the cilia appear well extended and very conspicuous owing to their dark colour. The inclusions (*inc.*) and basal granules (*b.gr.*) are well defined. Mucigen granules appear a dense black. Intracellular fibrillae (*int.f.*) are demonstrated but their course is not easily followed.

The intracellular fibrillae are best demonstrated in these cells by fixation in Duboscq-Brasil, after which their course within the cell is easily followed.

Staining in Meyer's mucicarmine colours the mucus glands (*m.c.* and *g.m.c.*) a deep red or purple, showing that their secretion is quite definitely mucin.

After Mallory's triple stain a shallow zone of cytoplasm (*sh.z.*), immediately below the basal granules of the ciliated cells, takes up a characteristic pale blue colour, while the remainder of

the cytoplasm stains purplish. The corresponding zone, after staining in safranin, appears pinkish, the rest of the cytoplasm takes up little or no stain.

Osmic fixation, by virtue of its less violent action, is by far the best fixative for this epithelium, for what is lost in lack of clearness of the intracellular fibrillae is more than compensated for by the retention and clear differentiation of the cell inclusions. The mucigen granules of the mucus glands, and the irregular bodies of the granulose layer (*gr.l.*) of the ciliated cells, blacken with osmic acid and consequently show very distinctly.

Duboscq-Brasil, on the other hand, though showing basal granules and intracellular fibrillae very clearly, tends to dissolve the cell inclusions and cause retraction of the cilia.

The ciliated cells are most predominant. They are tall and columnar, about 40μ high and 6μ across and separated by distinct boundaries. The nucleus (*n.cl.c.*) is oval, basal, and possesses scattered irregular chromatin masses, a distinct nuclear membrane, and sometimes a nucleolus is visible. Often, as is quite common in similar epithelia, more than one nucleus may be present in a cell.

The mucus cells (*m.c.*) are much less numerous. They are vesicular in form, and appear as a series of small sacs sunk within the epithelium. Their secretion is extruded as a discrete granular goblet. The least predominant are the gland cells (*g.c.*). These are very inconspicuous, particularly if discharged, when they appear to form merely a thickened boundary between two ciliated cells. The cytoplasm may possess a few scattered granules, presumably precursors of the secretion which is extruded as a discrete hyaline globule (*sec.g.c.*).

Throughout the gut wandering cells are common. They may be seen in the lumen of the gut, in the epithelial cells themselves, in intra-epithelial spaces (*vac.ep.*), or in the subjacent tissues. They occasionally present evidence of being amoeboid. The cytoplasm varies enormously in its capacity to take up various stains. After osmic fixation the cytoplasm may refuse to take up stain and appears greyish with coarse blackish granules. In other cases the cytoplasm may show marked avidity for stains like safranin, Altmann's acid fuchsin and Delafield's haematoxylin, and the cells appear merely as deeply staining bodies devoid of any obvious structure. Sometimes the cytoplasm may take up stains such as safranin and light green, or Mallory, differentially. Very frequently the cytoplasm is vacuolated. The nucleus, when visible, may be spherical, oval, or crescentic, with small scattered chromatin masses.

The epithelium rests on a basement membrane (*b.m.*) staining deeply in light green, the fuchsin of Champy-Kull, and the aniline blue of Mallory. This overlies a layer of circular smooth muscles (*circ.m.*). Giant mucus cells (*g.m.c.*), sometimes measuring as much as 32μ across, occur in the submucosa; their cytoplasm gives the characteristic reaction with mucicarmine, and their nuclei (*n.m.c.*) are ovoid, with small scattered chromatin granules.

Staining with Mallory revealed no such structures as the intraepithelial canals described by MACKINTOSH (1925) in *Crepidula*, YONGE (1926b) in *Ostrea*, and GRAHAM (1932) in *Patella*.

2—*The Oesophagus, Section B.* Fig. 7 depicts a transverse section of the gut wall of this region, and was drawn from a preparation that had been fixed in Flemming without acetic and stained in safranin and light green. The epithelium lining this region of the oesophagus contrasts sharply with that lining the preceding section. It exhibits a markedly ragged appearance associated with the great predominance of mucus cells, and due to the irregular masses of secretion (*sec.g.c.*), produced by these cells, and to the spaces left in the epithelium by the discharged goblets of mucin (*dis.g.*).

These goblets appear very conspicuous after such stains as light green or Meyer's mucicarmine, and after fixation in Flemming without acetic they are seen to be loaded with coarse granules of mucigen which blacken with osmic acid. The remainder of the cytoplasm of the mucus cells contains diffusely scattered granules, some of which (*bl.gr.*) are very small, irregular, and blacken with osmic acid, others (*g.gr.*) are larger, more regular, and appear greyish after osmic fixation. Vacuoles of varying sizes are present; the small ones (*s.v.*) are inconspicuous and appear to be devoid of inclusions, but the large ones (*l.v.*) are very conspicuous and contain

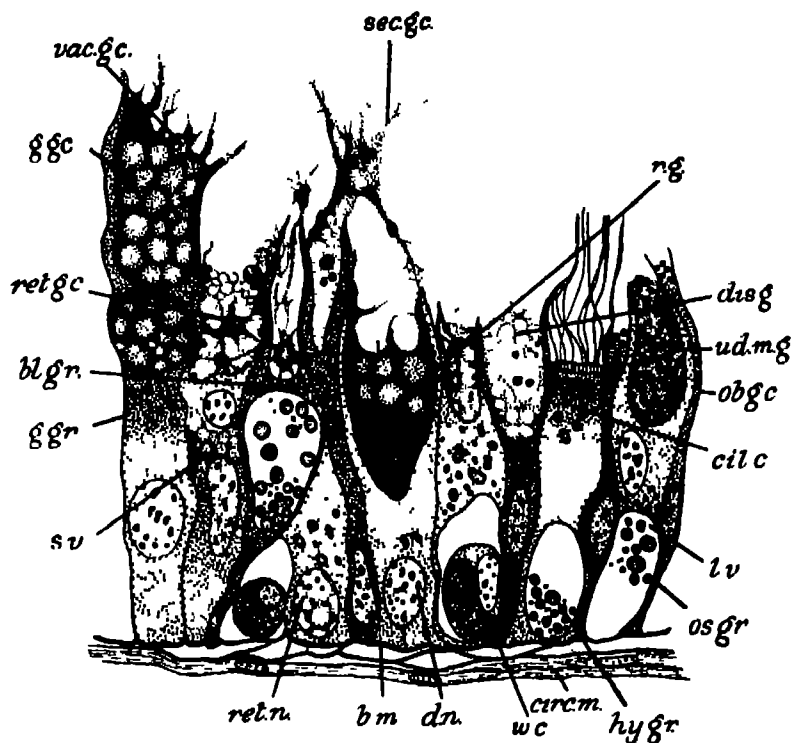


FIG. 7.—Portion of a transverse section through the wall of Section B of the oesophagus. Fixed Flemming without acetic; stained safranin and light green. *b.m.* basement membrane; *bl.gr.* irregular granules; *cil.c.* ciliated cell; *circ.m.* circular muscle; *dis.g.* cavity left by discharge of goblet; *d.n.* nucleus with diffuse chromatin granules; *g.gc.* mucigen granules remaining behind in discharged goblet; *g.gr.* granules appearing greyish after osmic fixation; *hy.gr.* hyaline granules; *l.v.* large vacuole; *ob.g.c.* shrunk gland cell; *os.gr.* granules appearing blackish after osmic fixation; *r.g.* undischarged portion of goblet; *ret.g.c.* reticulum remaining in discharged goblet; *ret.n.* nucleus with reticular structure; *s.v.* small vacuole; *sec.g.c.* secretion of goblet cell; *ud.m.g.* undischarged goblet of mucigen; *vac.g.c.* vacuole in goblet; *w.c.* wandering cell. $\times 1330$.

spheroids about 2μ in diameter which vary in their reactions to stains and fixatives, for while some (*hy.gr.*) stain in light green and appear hyaline, others (*os.gr.*) take up safranin and blacken with osmic acid to a varied extent.

The nucleus is ovoid, with a distinct nucleolus staining light red in safranin and Altmann's acid fuchsin; the chromatin may appear in the form of irregular scattered granules (*d.n.*) or in the form of a reticulum (*ret.n.*).

Their secretion consists of the whole, or part of one of the goblets (*ud.m.g.*) described above, but usually owing to the pressure of food in the lumen of the gut, and the action of the ciliated

cells, the mucin goblets become drawn out into irregular strands of secretion (*sec.g.c.*). The discharge of secretion leaves behind a cavity in the cell (*dis.g.*) which contains either a sparse reticulum (*ret.g.c.*) or a denser vacuolated mass of undischarged secretion (*vac.g.c.*) with adherent granules (*g.gc.*). When only a part of a goblet is discharged, the undischarged portion (*r.g.*) remains in the bottom of the cavity.

Cells which have been secreting for a considerable time become much shrunken, and frequently almost obliterated (*ob.g.c.*) by the pressure of the surrounding secreting cells.

Ciliated cells, precisely the same in structure as those described for section A, occur scattered throughout section B. The wandering cells (*w.c.*), though having the same structure and distribution as those described in the preceding section, are here much more numerous.

The tissues of the submucosa are the same as those described for the preceding section.

Compared with the epithelium of the buccal mass, that of the oesophagus is conspicuously different in possessing no hard chitinous covering and a relatively scanty muscular coat. This is correlated with a complete difference of function. The distribution of ciliated and mucus cells in the oesophagus accords excellently with its functioning. In section A, which is nearest the buccal mass, the predominance of ciliated cells is correlated with a need for the rapid transference of small food particles thrust up, on the radula (see p. 198), into the oesophagus. The scattered mucus cells produce a viscid secretion which appears to serve two purposes; first in cementing small food particles into strings, and secondly in acting as a protection for the delicate epithelium, against the numerous projecting sponge spicules which are thrust up with the triturated food on the radula. This latter conclusion is supported by the observation that the amount of mucus secreted seems greater than would be required merely to cement food particles into strands.

By the time the food particles enter into section B of the oesophagus, they are already segregated into food strands, and consequently the chief function of this region is to ensure the stability of these strands by admixture with abundant mucus. Hence the primarily important mucus cells are very predominant.

3—*The Caecum* (for anatomy see p. 178)

Fig. 8 is a transverse section of the wall of the caecum as seen after fixation in Flemming without acetic and staining in safranin and light green.

The epithelium lining the caecum is conspicuous by its large number of ciliated cells, which differ from those previously described, in being smaller, having large nuclei, and very sparse cytoplasmic contents. Owing to the latter feature it is possible to see an extremely well-defined system of intracellular fibrils within the cell.

These cells are fairly regular in outline and are shortly columnar. They have large vacuoles (*vac.*) and the cilia end in distinct basal granules (*b.gr.*), which are prolonged within the cell into intracellular fibrils (*int.f.*).

The basal granules overlie a narrow clear zone of cytoplasm (*cl.z.*), which in turn overlies a deeper zone with irregular solid inclusions (*ir.g.*) appearing greyish or, more rarely, black, after osmic acid.

Deeper in the cell, in the region of the nucleus, regular ovoid inclusions (*ov.b.*), which take up safranin or light green, are present.

The nuclei (*n.cil.c.*) are ovoid or spherical, having a well-marked nuclear membrane and a very prominent nucleolus (*ncl.cil.c.*), which stains a characteristic bright red in safranin.

Between the ciliated cells small gland cells (*g.c.*) are present; these are conspicuous on account of their dense cytoplasm which appears a deep grey or brown after osmic fixation and which may contain a number of small dark granules (*gr.*). The nucleus (*n.g.c.*) never shows a reticular structure, has a distinct nucleolus and small irregular chromatin masses. These cells produce a secretion in the form of discrete globules (*sec.gl.*) staining bright red after safranin. The function of this secretion is discussed later.

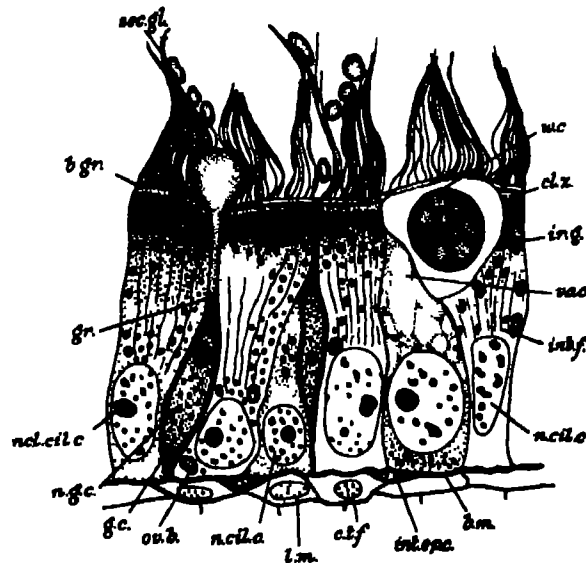


FIG. 8—Portion of epithelium of wall of diverticulum. Fixed Flemming without acetic; stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *cl.z.* clear zone beneath basal granules; *c.t.f.* connective tissue fibre; *g.c.* gland cell; *gr.* granule in neck of gland cell; *int.ep.c.* structure resembling intra-epithelial canal (see text); *int.f.* intracellular fibril; *ir.g.* solid inclusions appearing greyish after osmic acid; *l.m.* longitudinal muscle; *n.cil.c.* nucleus of ciliated cell; *n.g.c.* nucleus of gland cell; *ncl.cil.c.* nucleolus of ciliated cell; *ov.b.* ovoid inclusion; *sec.gl.* secretion of gland cell; *vac.* vacuole in ciliated cell; *w.c.* wandering cell. $\times 1500$.

Wandering cells (*w.c.*) are common throughout the epithelium.

For the demonstration of cell inclusions, Flemming's fluid proved by far the best fixative.

The intracellular fibrils tend to be hidden by cell inclusions, and hence they are not well demonstrated after fixation in Flemming, but they appear very clearly after fixing in Susa, when they can be traced from the basal granules, through the cell, to a point of insertion on the nuclear membrane.

The structure of the basement tissues yields a valuable clue for determining the method of function of the caecum (see p. 211). The epithelium rests on a conspicuous basement membrane (*b.m.*) which overlies connective tissue permeated by fibres (*c.t.f.*), and in the fundus and neck regions of the caecum, by a few longitudinal (*l.m.*) and circular muscles. In the head region, however, the muscular coat is greatly increased,

and here also, there is a greater development of small canals (*int.ep.c.*) between the bases of the epithelial cells. These canals contain a coagulum in which no structure is recognizable but which stains a bright red in Mallory. Their significance is unknown; they may possibly be a strengthening device, comparable with the intraepithelial canals described by MACKINTOSH (1925) for the style sac of *Crepidula*, by YONGE (1926*b*) for the style sac of *Ostrea*, and by GRAHAM (1932) for the midgut of *Patella*. Such strengthening devices occur where an epithelium is subjected to a considerable stress, e.g. in the style sacs noted above, and here such a function would accord with the well-developed muscular layer of the region, and the suggestion (see p. 212) that the gut wall is here chiefly concerned in moulding and ejecting a faecal bolus.

Certain of the ciliated cells described above present the peculiar appearance shown in fig. 26. Their free ends become expanded into globular masses (*ex.gl.*) which appear finely granular and stain a bright pink in Mallory, contrasting sharply with the rest of the cell, which stains purplish.

The expansions become abstricted as free rounded bodies which pass into the lumen of the caecum and have also been seen in the cavities of the mid- and hindgut.

These bodies cannot be droplets of secretion, since they are seen to retain their definite shape. Their appearance and mode of formation recalls that of the "ballots d'excrétion" described by DARBOUX (1900), and noted later by FORDHAM (1925), which are produced by abstriction from the cells lining the intestinal caeca of *Aphrodite aculeata*. Possibly like them, they form a means of excretion, being ejected from the caecum, together with the faecal masses produced there (see p. 212), and carried into the hindgut.

4—*The Digestive Gland* (for anatomy see p. 178)

Fig. 9 represents a portion of a transverse section of one of the tubules of the digestive gland, drawn from a preparation fixed in Flemming without acetic and stained in safranin and light green.

The epithelium is extremely variable in height, and is composed of cells of varied shapes. These cells appear to be ingestive, since they show large areas either loaded with inclusions or densely packed with vacuoles. This observation is confirmed by the results of iron-saccharate feeding (see p. 208). The free surface of the cells is variable in appearance. Sometimes it is produced into irregular cytoplasmic processes (*cy.p.*) which appear capable of ingesting solid particles. Elsewhere phagocytes may be abstricted from the surface, while at other times the epithelium may possess a striated border, like the cells of the digestive gland of *Patella* (GRAHAM 1932). When living tissue is examined, many of the liver cells may be seen to bear cilia.

As in other regions of the epithelium, Flemming without acetic proved to be by far the best fixative. It does not produce swelling nor dissolution of the cell inclusions, it clearly differentiates various regions of cytoplasm such as the homogeneous (*hom.a.*), and granulose (*gr.a.*) areas seen in fig. 9, and does not tend to cause retraction of the pseudopodial processes of the

cells. Duboscq-Brasil and Susa, on the other hand, tended to produce distortion of the cells, accompanied by retraction of the pseudopodial processes, and a certain amount of dissolution of the contents.

The cilia observed in living tissue only appeared in limited regions of preparations fixed in Susa, and indications of cilia were never found after fixation in Flemming without acetic. The free border of the cells may appear striated after fixation in Duboscq-Brasil. This appearance is frequently seen in fixed ciliated cells and the rare appearance of cilia in fixed preparations is

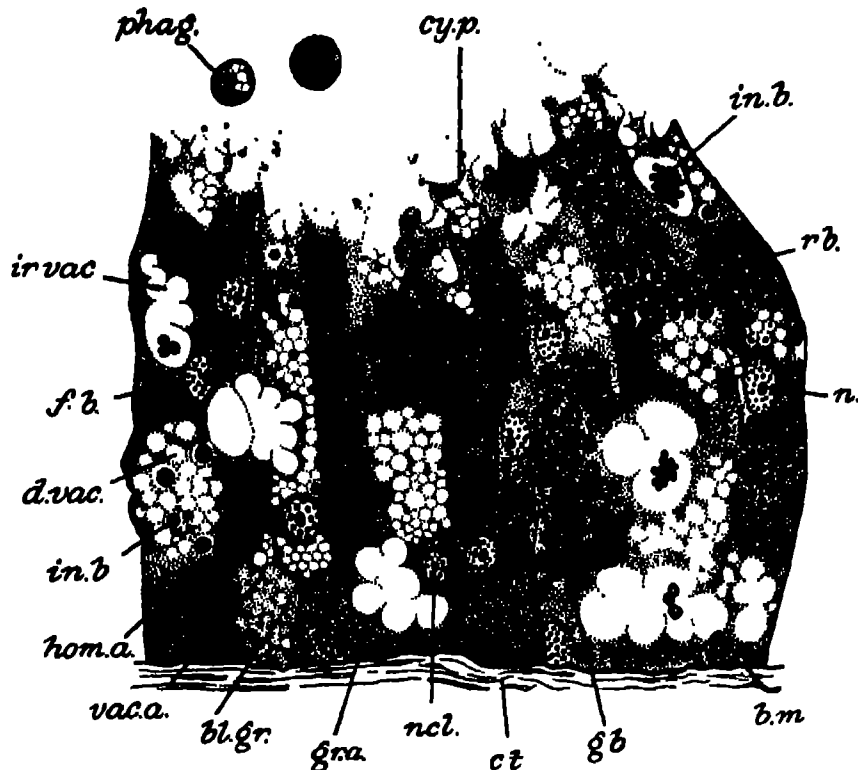


FIG. 9.—Portion of epithelium lining tubule of digestive gland. Fixed Flemming without acetic, stained safranin and light green. *b.m.* basement membrane; *bl.gr.* granules, appearing black after osmic, between vacuoles; *ct.* connective tissue underlying epithelium; *cy.p.* cytoplasmic process; *d.vac.* discrete vacuole; *f.b.* fatty body; *g.b.* solid inclusion staining in light green; *gr.a.* granular area; *hom.a.* homogeneous area; *in.b.* ingested body; *ir.vac.* irregular vacuole; *n.* nucleus; *ncl.* nucleolus; *phag.* phagocyte; *r.b.* inclusion appearing reddish after safranin; *vac.a.* vacuolated area. $\times 1600$.

not surprising when the results gained by other workers are considered. Thus PORTS (1923) states that in *Teredo* the cilia invariably found lining the liver tubules in the living animal, always fall off on fixation; while YONOE (1926a), working on the liver of *Nucula* and filibranchs, states that cilia can never be seen in sections of fixed material, though they have been observed in the living animals, and that a striated border to the cells is all that remains after fixation to indicate the former presence of cilia.

Many of the cells have highly vacuolated cytoplasm; the vacuoles may be small and discrete (*d.vac.*), or several such vacuoles may run together to form large irregular vacuoles (*ir.vac.*). The vacuoles frequently contain ingested bodies (*in.b.*), and in living tissue many are seen to contain

a clear refractive liquid, others have brownish contents, and some contain a greenish liquid. Almost every cell contains a large number of inclusions, of any size up to 4μ , many being surrounded by vacuoles.

The inclusions react to stains and fixatives in a very varied manner; some blacken in osmic (*f.b.*) and are presumably of a fatty nature, others take up safranin (*r.b.*), eosin or light green (*g.b.*), while after staining in Mallory some of the inclusions appear red, some blue, some purple, and some green.

Cytoplasmic differentiation is very marked, some areas (*gr.a.*) appearing coarsely granular, others free from granules, while other areas appear deep brown after osmic fixation, and either homogeneous (*hom.a.*), or vacuolated (*vac.a.*), with blackish granules (*bl.gr.*) between the vacuoles.

The nuclei (*n.*) are spherical or ovoid, variable in position, and have a distinct nucleolus (*ncl.*) staining bright red in safranin, and chromatin masses which appear small, scattered and indistinct after fixation in Flemming without acetic, but large and well defined after fixation in Susa.

The epithelium rests on a basement membrane (*b.m.*) overlying connective tissue (*c.t.*), which is devoid of muscle fibres and mucus cells, and which separates adjoining tubules from one another and from the overlying gonad.

The variable appearance of the free surface of the epithelium suggests that a phase change occurs. This suggestion is supported by the following facts.

First, several series of sections have been examined in which no phagocytes whatever are visible in the liver tubules.

Secondly, the extremely infrequent appearance of cilia lining the liver tubules in fixed material, while in the fresh material examined they were constantly present, suggests that the cilia may be readily withdrawn.

Thirdly, the free border of the cells is not always thrown out into irregular cytoplasmic processes, frequently it is a smooth uniform surface.

Similar phase changes have been described by other authors. POTTS (1923) working on *Teredo* states with regard to the epithelium lining the liver tubules: "I think that the epithelium passes through phases, and that ciliar retraction is followed by the putting out of pseudopodia and ingestion of wood. In such places there is also a multiplication of the nuclei accompanying assimilation, and separation of uninucleate phagocytic cells." Two other cases of phase change have been described by Miss GREENWOOD (1888, 1892). The first described occurs in the cells lining the archenteron of *Hydra*, where the cells may retract their cilia and form pseudopodia. The second is cited to occur in certain cells lining the gut of *Lumbricus*, where retraction of cilia is followed by a phase in which ingestion of fat occurs, the cells exhibiting a striated or rodged border.

The Phagocytes. When the tubules of the digestive gland of *Jorunna* are examined microscopically, numbers of free amoeboid cells may usually be observed. These cells have the appearance of phagocytes, many having within them vacuoles containing ingested foreign bodies. They are largely confined to the lumen of the digestive gland tubules, fig. 10, the caecum, and the midgut.

Similar cells were observed in three other nudibranchs, viz. *Goniodoris nodosa* (MONTAGU), *Archidoris britannica* (JOHNSTON), and *Polycera quadrilineata* (O. F. MÜLLER). This is of interest, since YONGE (1926a) states that phagocytes are not found in the gut of Gastropoda.

The phagocytes of *Jorunna* were examined in preparations made by teasing out the digestive gland tubules of living specimens, and also in sections of fixed material. Several are shown in figs. 10, 14, 22, 23 and 24.

Flemming without acetic, followed by safranin and light green, gave excellent results, nuclear detail and cytoplasmic inclusions were well preserved, fatty substances were demonstrated, and delicate structures such as small vacuoles were only slightly distorted.

Other fixatives such as Duboscq-Brasil and Susa were not so satisfactory, since they tended to dissolve cell inclusions and to cause distortion of vacuoles, though the former fixative proved useful when used in conjunction with Mallory's triple stain, which does not stain satisfactorily after Flemming without acetic, in picking out the various contents of the vacuoles in distinct colours.

The phagocytes, as seen after fixation, are very variable in size (varying from 5 to 24 μ), and assume various shapes, the most common being spherical.

Each cell (fig. 14) has a single conspicuous ovoid nucleus (n.) precisely similar to those described for the digestive gland cells. The cytoplasm is like that of the digestive gland cells. Frequently it is highly vacuolated, the vacuoles containing inclusions which take up safranin or light green with avidity, or fatty substances appearing greyish, deep brown, or black after osmic acid. Fatty granules frequently occur scattered through the cytoplasm. In the living cells the cytoplasm is seen to be colourless and usually hyaline, with a few vacuoles containing oil-like globules.

Often when living tissue is examined, the liver tubules are seen to be infested with ciliates. It was thought at first that these organisms when fixed, or in certain phases when alive, might assume the rounded form described above for the phagocytes. That the cells described above as phagocytes are really so, and not in any way connected with these ciliates, is shown by the following.

No cilia could be seen on the cells in question with the exception of a few isolated instances (see below), where the cells bore irregularly disposed clumps of cilia. In these instances, the cells were easily distinguishable from the ciliates since their motion was irregular, jerky and indeterminate.

Again, as noted above, the nuclei of the cells in question resemble those of the liver cells in every way and, moreover, their size, when measured with a micrometer scale, was seen to vary within exactly the same limits as the nuclei of the liver cells.

The latter observation, in addition to providing evidence distinguishing between the cells described as phagocytes and the ciliates, forms a valuable clue to the origin of these cells, which is supported by further evidence.

First, both digestive gland cells and phagocytes are capable of ingesting solid bodies at their free surface, p. 208.

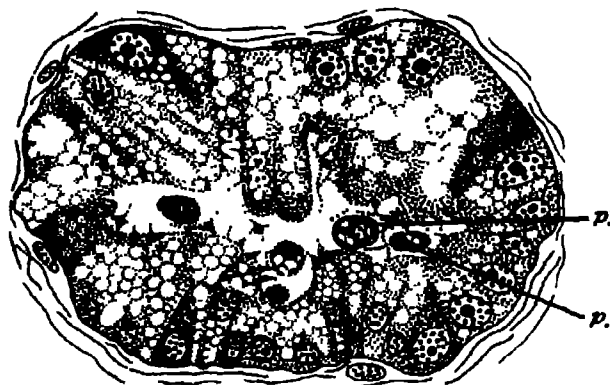


FIG. 10



FIG. 13

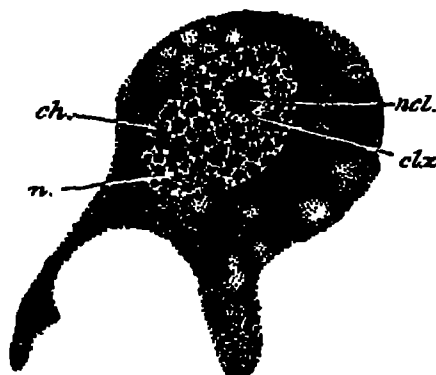


FIG. 14

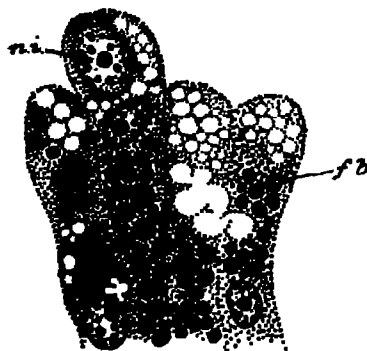


FIG. 11

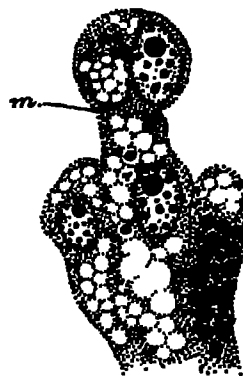


FIG. 12

FIG. 10—Transverse section of tubules of the digestive gland showing phagocytes in the lumen. Fixed Flemming without acetic, stained safranin and light green. $\times 450$.

FIG. 11—First stage in the abstriction of a phagocyte. $\times 1500$.

FIG. 12—Second stage in the abstriction of a phagocyte. $\times 1350$.

FIG. 13—Third stage in the abstriction of a phagocyte. $\times 1850$. All from *Jorunna*. Fixed Flemming without acetic, stained safranin and light green.

FIG. 14—A large phagocyte from the lumen of the midgut, fixed Flemming without acetic, stained safranin and light green. $\times 2700$.

ch. chromatin; *cl.z.* clear zone around nucleolus; *f.b.* fatty body; *m.* membrane (see p. 194); *n.* nucleus; *n.i.* daughter nucleus (see text, p. 194); *ncl.* nucleolus; *p.*, *p'.* phagocytes.

Secondly, when living material is examined, phagocytes bearing irregularly disposed groups of cilia may occasionally be seen. No cilia were ever observed on the cells seen in fixed material. As noted on p. 190, the free border of the digestive gland epithelium may often be seen to bear cilia which tend to disappear on fixation.

These close similarities lead to the conclusion that the phagocytes are derived from the epithelium of the digestive gland. This is confirmed by the appearance of the free border of the epithelium, from which uninucleate cells, identical in appearance with the phagocytes, may occasionally be seen to be abstricted. The stages of the process are shown in figs. 11, 12 and 13, and seem to be as follows:

1 (fig. 11)—A nucleus at the tip of a cell has divided, one of the daughter nuclei (*n.i.*) passes into the slightly swollen apical region.

2 (fig. 12)—The nucleus and the surrounding zone of cytoplasm appears to become separated from the remainder of the cell by a fine membrane (*m.*).

3 (fig. 13)—Separation of the nucleus and surrounding cytoplasm from the tip of the cell as a phagocyte.

Hence the occasional appearance of irregular groups of cilia on phagocytes, which seems at first a very unusual phenomenon, is easily explicable by assuming that the cells are cut off whilst the digestive gland epithelium still bore cilia, and before the change from the ciliated phase, to that in which pseudopodia are put out, and phagocytes are abstricted, was complete.

PORRS (1923) has described cells of the digestive gland of *Teredo* as giving rise to similar phagocytes in the same manner.

5—*The Midgut* (for anatomy see p. 177)

The midgut is lined with a very regular epithelium composed of two types of cell.

The most predominant cells are ciliated. They closely resemble those described for the caecum (p. 187).

The other type of cell occurs in very small numbers and is a goblet mucus cell. It is identical with the type described for the oesophagus section B.

The epithelium rests on a basement membrane, below which is a zone of connective tissue permeated by circular, longitudinal, and oblique muscle fibres, and containing large mucus cells. The latter resemble those which occupy a similar position in the oesophagus.

6—*The Hindgut* (for anatomy see p. 178)

Like the oesophagus, the hindgut may be subdivided into sections A and B, on the basis of different histological characters of the lining epithelium.

1—*The Hindgut, Section A.*

Fig. 15 represents a portion of a transverse section of the wall of this region of the hindgut. The fixation and staining was precisely the same as that employed in the preparations figured previously.

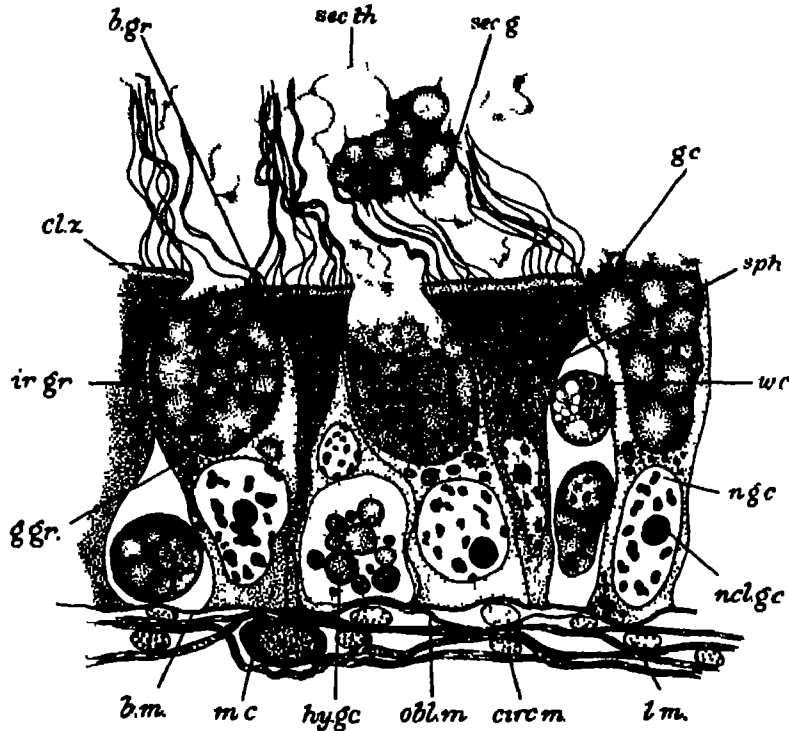


FIG. 15—Portion of a transverse section of the wall of section A of the hindgut. Fixed Flemming without acetic, stained safranin and light green. *b.gr.* basal granules; *b.m.* basement membrane; *circ.m.* circular muscle; *cl.z.* clear zone underlying basal granules; *g.c.* goblet cell; *g.gr.* granules appearing greyish after osmic fixation; *hy.gr.* hyaline granule; *ir.gr.* irregular granules; *l.m.* longitudinal muscle; *m.c.* mucus cell in submucosa; *n.g.c.* nucleus of gland cell; *ncl.g.c.* nucleolus of gland cell; *obl.m.* oblique muscle; *sec.g.*, *sec.th.* secretion of mucus cells (see text); *sph.* spheroid appearing greyish after osmic fixation; *w.c.* wandering cell. $\times 2000$.

The chief feature of the epithelium is the prominence of the goblet mucus cells.

These strongly resemble the corresponding cells of section B of the foregut and need not be described here. Their secretion is discharged as a discrete goblet (*sec.g.*) which becomes more or less whipped out into threads of secretion (*sec.th.*) by the action of cilia of surrounding cells.

The intermingled ciliated cells are irregular in outline and usually shrunken in appearance. The cilia end in basal granules which are not nearly as distinct as in other parts of the gut, and which overlie a narrow strip of cytoplasm (*cl.z.*). This, since it has no obvious inclusions, contrasts strongly with the underlying cytoplasm, which appears denser and is loaded with granules. The cytoplasm of these cells sometimes appears highly vacuolated, sometimes homogeneous and finely granular, but more usually it is seen to contain large numbers of irregular, indistinct granules (*ir.gr.*) and occasionally one or two large spheroids (*sph.*) appearing greyish after osmic. Intracellular fibrils may occasionally be seen running through these cells, and in one or

two cases could be traced to the nuclear membrane. The nucleus is large, basal in position, has a prominent nucleolus and large chromatin granules, not forming a reticulum. Wandering cells (*w.c.*) like those described for other parts of the gut are very numerous here.

2—The Hindgut, Section B.

Portion of a transverse section of the gut wall of this region, fixed and stained as before, is shown in fig. 16.

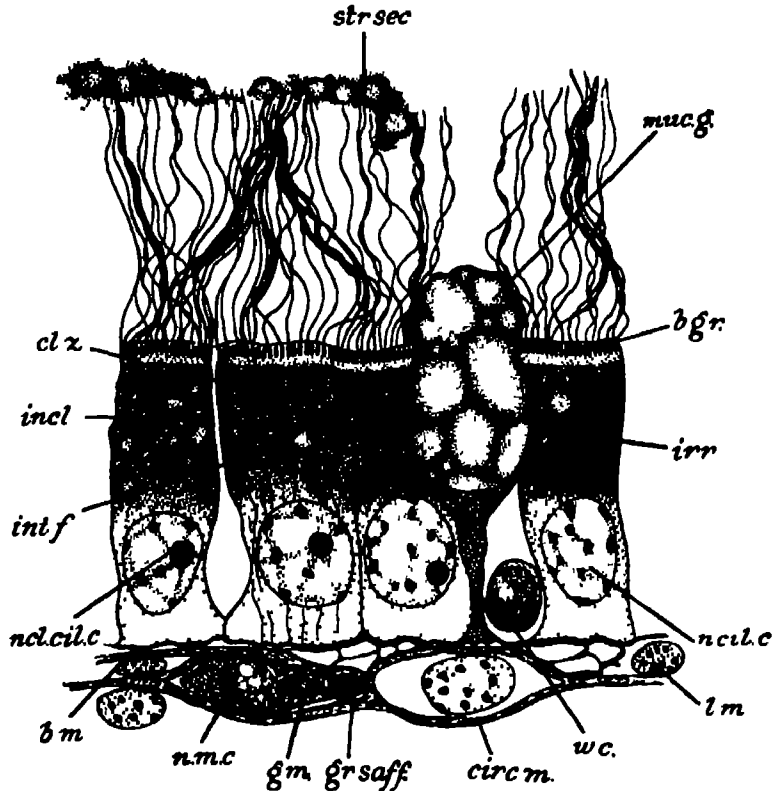


FIG. 16—Portion of transverse section of wall, section B of hindgut. Fixed Flemming without acetic, stained safranin and light green. *g.m.* goblet of mucin in mucus cell of submucosa; *gr.saff.* granules in mucus cell, staining with safranin; *int.f.* intracellular fibrils; *irr.* irregular inclusions; *muc.g.* goblet of mucus; *n.m.c.* nucleus of mucus cell; *str.sec.* string of secretion. Other letters as in previous figures. $\times 2000$.

The dominant feature of this epithelium is its extreme regularity. The cells have a uniform height of about 20μ and the basal granules (*b.gr.*), clear zone (*cl.z.*), and granular zone, of the ciliated cells, form three very distinct layers, giving the epithelium a markedly characteristic appearance. After staining in safranin and light green, as a result of the even distribution of the granules in the ciliated cells (*irr.* and *incl.*), the upper halves of these cells stain green, while the lower halves appear red.

The epithelium is composed of the same two types of cell as the preceding section, but here, in sharp contrast with section A, it is the ciliated cells which are most numerous.

These cells possess extremely long cilia ending within the cell in very distinct basal granules (*b.gr.*), overlying a strip of clear cytoplasm (*cl.z.*), and which can be seen in certain cells to be

prolonged into intracellular fibrils (*int.f.*). The cytoplasm of the upper half of the cell appears rather denser than that of the lower half, which houses the nucleus. It contains large numbers of regular solid inclusions (*incl.*) which take up light green, with intermingled irregular bodies (*irr.*) appearing dark after osmic acid.

The nucleus (*n.cil.c.*), situated in the less dense cytoplasm of the lower half of the cell, is usually almost spherical and has a very distinct nuclear membrane, a prominent nucleolus (*ncl.cil.c.*), and chromatin granules forming the nodes of a nuclear reticulum.

The mucus cells are precisely similar to those of preceding sections. Their secretion is extruded as a goblet, which becomes whipped out into strands (*str.sec*) by the cilia of adjoining cells.

Wandering cells (*w.c.*), having precisely the same structure and disposition as in other parts of the gut, occur here, but they are less numerous than in the epithelium of the preceding section A.

The epithelium rests on a basement membrane (*b.m.*) which is prominent after the Champy-Kull stain, when it takes up a deep red colour, and after Mallory when it stains bright blue. Beneath the basement membrane is connective tissue permeated by oblique, longitudinal (*l.m.*), and circular (*circ.m.*), muscle fibres. Large mucus cells (*m.c.*, fig. 15) occur in the connective tissue of the submucosa. These are irregular in outline, and contain large numbers of granules which take up safranin (*gr.saff.*), and one or more goblets of mucin (*g.m.*). Their nuclei (*n.m.c.*) are spherical, having a prominent nucleolus and chromatin in the form of a reticulum.

The hindgut resembles the oesophagus in possessing a lining of ciliated and mucus cells. This histological resemblance is correlated with a functional one, for whereas the oesophagus was concerned with cementing particles into food strands, and transferring the latter to the midgut and liver, the hindgut is concerned with aiding in the cementing of faecal particles to form a compact bolus, and the transference of this bolus to the anus.

It is interesting to note that in section A, where the need for cementing up stray faecal particles ejected from the digestive diverticula (see p. 213) is greatest, the predominance of mucus cells is very marked.

VI—FEEDING

Movement of the Radula

The feeding movements can be observed by watching the movement of the mouth in animals creeping upside down on the surface film of the water, or on the glass sides of an aquarium.

During the feeding process, the buccal mass is erected, so that the outer lips, which are depicted in fig. 2 pointing forwards, are pressed closely against the substratum on which the animal is crawling. As the cycle of movement begins, the closely apposed outer lips begin to move apart, thus exposing the inner lips (*i.l.* fig. 2) which protrude between them. The inner lips now begin to part, the process beginning in the middle and spreading outwards. This exposes the buccal lips (*b.l.* fig. 2).

Meanwhile the odontophore (*od.*) is being thrust ventrally, so that it comes to fill

the area enclosed by the buccal lips. As its progress continues, the buccal lips are forced apart until they form merely a narrow rim around the protruded odontophore. As a result of these changes, the radula (*rad.*) has been carried down so that its teeth lie in contact with the substance on which the animal desires to feed. A rapid forward and upward movement of the odontophore now occurs, causing it to be withdrawn into the buccal cavity. Simultaneously, there is a deepening of the median longitudinal groove which separates the two lobes of the radula, so that each of the more lateral rows of teeth are successively exposed.

Since the radula is being drawn upwards and forward, the whole structure is so tilted that all the rows of teeth outside the radula sac are brought to bear on the substratum.

The movement of the radula results in a scraping or rasping process, which tears up the substance across which the radula is drawn, and furthermore results in the pieces so torn being held on the ends of the recurved radula teeth. Consequently torn up shreds of food are constantly being transferred on to the ciliated ridges of the oesophagus each time the radula is carried forwards and upwards into the buccal cavity in completing its stroke.

On the withdrawal of the radula into the buccal cavity, the inner lips close together, and the cycle starts again. By taking the average time occupied by the various phases of the cycle, the following data has been obtained:

Phase 1. Parting of the inner lips	4 sec.
Phase 2. Protrusion of the odontophore	3 sec.
Phase 3. Upward and forward movement of the radula exposing all its functional teeth	4 sec.
Phase 4. Retraction of odontophore	3 sec.
Phase 5. Closing of inner lips	4 sec.

Movement of Particles in the Gut—I. By Cilia

Two methods were used for determining the direction of ciliary currents.

In the method of direct observation, fine particles of carmine were projected from a fine pipette on to pieces of excised gut in sea water. The ciliary currents were adduced from the movement of the carmine particles over the internal surface of the gut, observed by a binocular microscope.

The method of determining ciliary currents by the use of carmine particles has several marked disadvantages (see below). It occurred to me that it might be possible to deduce the direction of the beat of the cilia from their appearance in sections of fixed material.

GRAY (1930) has shown that a cilium during its forestroke is stretched out straight and, on recovery, bends forwards with the free tip pointing in the direction of its effective beat. Therefore, if in sections of fixed preparations, groups of cilia showed a

constant arrangement, uniformly bent at right angles with their free tips pointing in one direction, then it could be inferred that the cilia were producing a current in the direction of their free tips. This principle was used in determining the ciliary currents inside the gut. Whenever possible, these inferred results were checked against observations in living tissues using the carmine method of direct observation, and the evidence obtained from both methods was correlated and combined. The method of determining ciliary currents by the use of sections was suggested to me by Professor H. GRAHAM CANNON. It is novel and possesses two distinct advantages over the other method.

First, the detection of minute ciliary tracts is rendered much easier. The currents on the sides of the ridges in the oesophagus of *Jorunna* are extremely difficult to detect by the use of carmine particles, partly owing to the smallness of the ridges, and partly because the latter are so closely approximated as to prevent any but the most minute carmine particles penetrating between them.

Secondly, by this method, the cilia-beating surfaces are preserved in their normal spacial and morphological relations to one another. This is impossible when, using the carmine method, it becomes necessary to open out structures in order to put carmine on their internal surfaces. Thus, for example, the tops of the walls of a narrow groove may be normally adjacent, and their cilia may act in co-operation, but the operation of opening up the gut and pinning it out might separate widely these two regions.

It was found that the fixed tissues gave constant results. Cilia in any region are always found to be fixed in the same position with the sole exception of one particular groove of the gut where a ciliary reversal is indicated (see below).

No cases were found where the cilia were pointing haphazard, except when this appearance was associated with bad fixation, or the presence of abundant food particles, or mucus, in the lumen of the gut, and such cases were not used for determining the direction of currents.

Other instances, in which the beat of the cilia appeared anomalous when determined by this method, all proved to be explicable by some morphological peculiarity of the individual sections. The obliteration of one of the primary ridges of the oesophagus or hindgut causes the cilia on the sides of the ridge, which normally beat up towards the lumen of the gut, to appear as an isolated tract in the wall of the gut, composed of two groups of cilia which beat towards each other without apparent reason.

Critical fixation is absolutely necessary in order to determine ciliary currents in this manner, and though several fixatives were tried, it was not possible to secure critical fixation throughout the entire gut of a single specimen. By combining the evidence of several series, however, it was possible to obtain a complete picture of the ciliation of the whole gut. Duboscq-Brasil, Flemming without acetic and Susa fixatives gave reasonably satisfactory evidence for this method, but the most successful

preparations were fixed in a solution of 4 parts absolute alcohol and 1 part glacial acetic acid, for 1 hr., followed by several washes in absolute.

The two methods outlined above, when combined, form an excellent means of determining ciliary currents, for not only does one method serve to check the other, but the currents observed by the carmine particle method can be analysed in detail by the subsequent examination of sections. Thus a current observed by carmine may possibly be the resultant of several component currents acting on one another. Such complex currents are very readily resolved into their components by the method of using sections, which permits the direction of the beat of all the cilia to be determined easily.

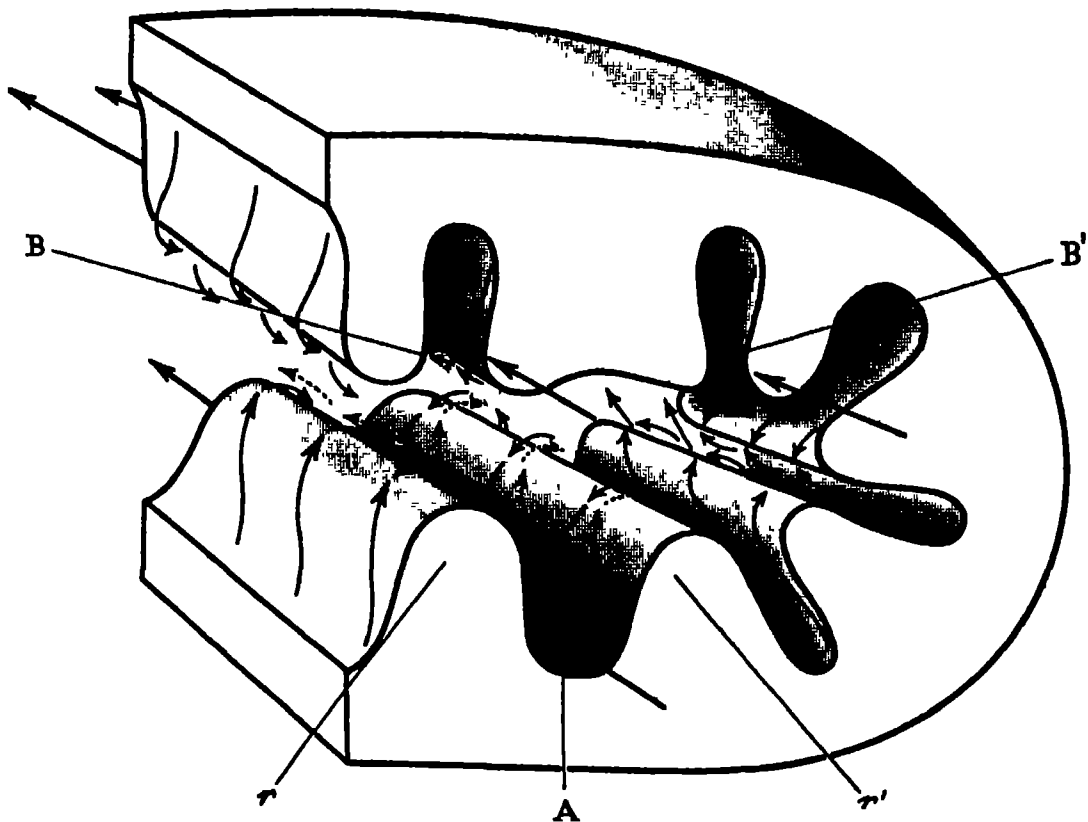


FIG. 17—Diagram showing the ciliary currents in the oesophagus and hindgut. The figure represents a thick slice from the middle of the oesophagus (or hindgut) viewed anteriorly from the right side and slightly from above. In order to give a clearer indication of the ciliary currents, part of the right aspect has been cut away. The secondary ridges are not shown. *A, B, B'* food tracts with food particles. *r, r'*, see text. Dotted arrows indicate ciliary reversal (see text). \times about 120.

Transference of Food Particles along the Oesophagus (see fig. 17)—As the odontophore is thrust into the oesophagus at the end of its cycle of movements, it comes into contact with the cilia on the lining ridges, which take up any food particles it may bear.

The majority of particles are taken up by the powerful cilia of the three tracts *A*, *B* and *B'* which beat along the oesophagus into the midgut. Any particles falling on the summit or sides of the ridges *r* and *r'* are swept obliquely downwards into the food tract *A*. The cilia on the summit of the remaining ridges fling particles obliquely backwards into the lumen of the gut, where they are taken up by the tracts *B* and *B'* either directly or after having been taken up by the cilia of a neighbouring ridge.

Particles which fall into any of the grooves, with the exception of that housing the food groove *A*, are carried obliquely backwards to the tops of the surrounding ridges by the cilia lining the sides, and are subsequently projected into the lumen of the gut by the cilia described above.

The secondary ridges (see p. 177) have no real significance in the feeding process and consequently may be disregarded.

The main result of these currents is that the food particles, mixed with mucus, which is secreted by the gut wall throughout the oesophagus (p. 187) and helps to bind the food particles together, pass into the midgut along the three main tracts *A*, *B* and *B'*.

When the animal is feeding actively, the amount of food present in the oesophagus is large enough to cause the food present in the three tracts to coalesce into one large mass of food, which is passed down the oesophagus as a compact bolus.

Neither sorting of food particles according to size, nor rejection of unwanted particles occurs.

The ciliary currents described above are primarily feeding currents and may be observed when the oesophagus contains a large number of particles. When the oesophagus is devoid of food particles, the cilia beating obliquely down the sides of the ridges *r* and *r'* into the food groove *A* reverse the direction of their beat so that they now beat out of the groove and obliquely backwards towards the summit of the ridges on either side. Thus the cilia on the sides of the ridges *r* and *r'* now beat in a similar direction to those on the sides of the remaining ridges, and the food groove *A* ceases to function as such. The ciliary currents now appear to be purely cleaning currents, excess mucus and any food particles which remain in the grooves are carried to the tops of the ridges and transferred to the tracts *B* and *B'* which carry them to the midgut.

The appearance of the cilia on the sides of the groove *A* indicates beyond doubt that the direction of their beat is reversible. Of the series of sections examined, some showed cilia with tips pointing out of the groove, others with cilia which pointed into the groove. In either case the tips of all the cilia lining groove *A* pointed uniformly in one direction or the other, which was constant throughout each series. There was no evidence of ciliary reversal in any of the other grooves.

The nature of the stimulus bringing about reversal has not been determined. It may be that contact between the wall of the oesophagus and masses of food brought up on the radula during the feeding process constitutes the stimulus for reversal. If

this be so, then the stimulus of food entering the oesophagus would cause the cilia in question, which, while the oesophagus was devoid of food, would be beating out of the groove (see above), to reverse the direction of their beat, and thus form an effective food channel.

Similar cases of ciliary reversal have been described before, in particular by YONGE (1930) in *Fungia*, where he describes how contact between food particles and the base of the tentacles brings about a reversal of the cilia on the oral disk.

Movement of Particles in the Midgut (see fig. 18)—As the food particles from the oesophagus enter the midgut, they are taken up by powerful currents circulating through the tubules of the digestive gland. These currents are maintained partly by the cilia lining the openings from the midgut into the digestive gland and partly by the cilia of the digestive gland cells.

Some of the cilia round the openings beat from the midgut into the digestive gland, while others beat in the reverse direction. In every animal examined, the openings of the digestive gland were beset with these two groups of cilia, though the exact disposition of the outward and inwardly beating cilia was found to vary in different individuals.

A similar disposition of cilia around the openings of the digestive gland tubules, maintaining a similar current, has been described by YONGE for lamellibranchs generally (1926*a*).

Owing to the anterior position of opening *I*, the majority of particles leaving the oesophagus enter the digestive gland by the inward current (*ent.c.*).

During the circulation through the digestive gland tubules most of the particles will probably be taken up by phagocytes and by the ingestive cells lining the tubules. Any particles which are not taken up, together with undigested particles which have been rejected by the phagocytes and liver cells, are returned to the midgut by the outward current (*ext.c.*). Here the above particles may be taken up by the spirally ascending current into the hindgut (*H.G.*), or they may be wafted into the caecum (*cae.*), or together with some further food particles passed in from the oesophagus, they may be passed through one of the other openings and circulated through another group of digestive gland tubules. Thus there is set up a mechanism whereby a continuous circulation of food particles through the tubules of the digestive gland is maintained, combined with a mechanism for the rejection of unwanted particles in the caecum (p. 211) and hindgut.

The walls of the midgut are covered with cilia, producing the cleaning currents indicated by the small arrows in fig. 18. These currents are in two groups, the dorsal, which beat directly into the hindgut, and the ventral, which transfer particles to a ventral tract of longitudinally beating cilia. This latter tract, in addition to keeping the floor of the midgut clear of particles and mucus, and hence preventing clogging of the mechanism, ensures that particles which have either fallen out of circulation, or

which on leaving the oesophagus were not taken up by the current (*ent.c.*), are carried to a region where they are likely to meet the powerful currents around the openings of the remaining digestive gland tubules.

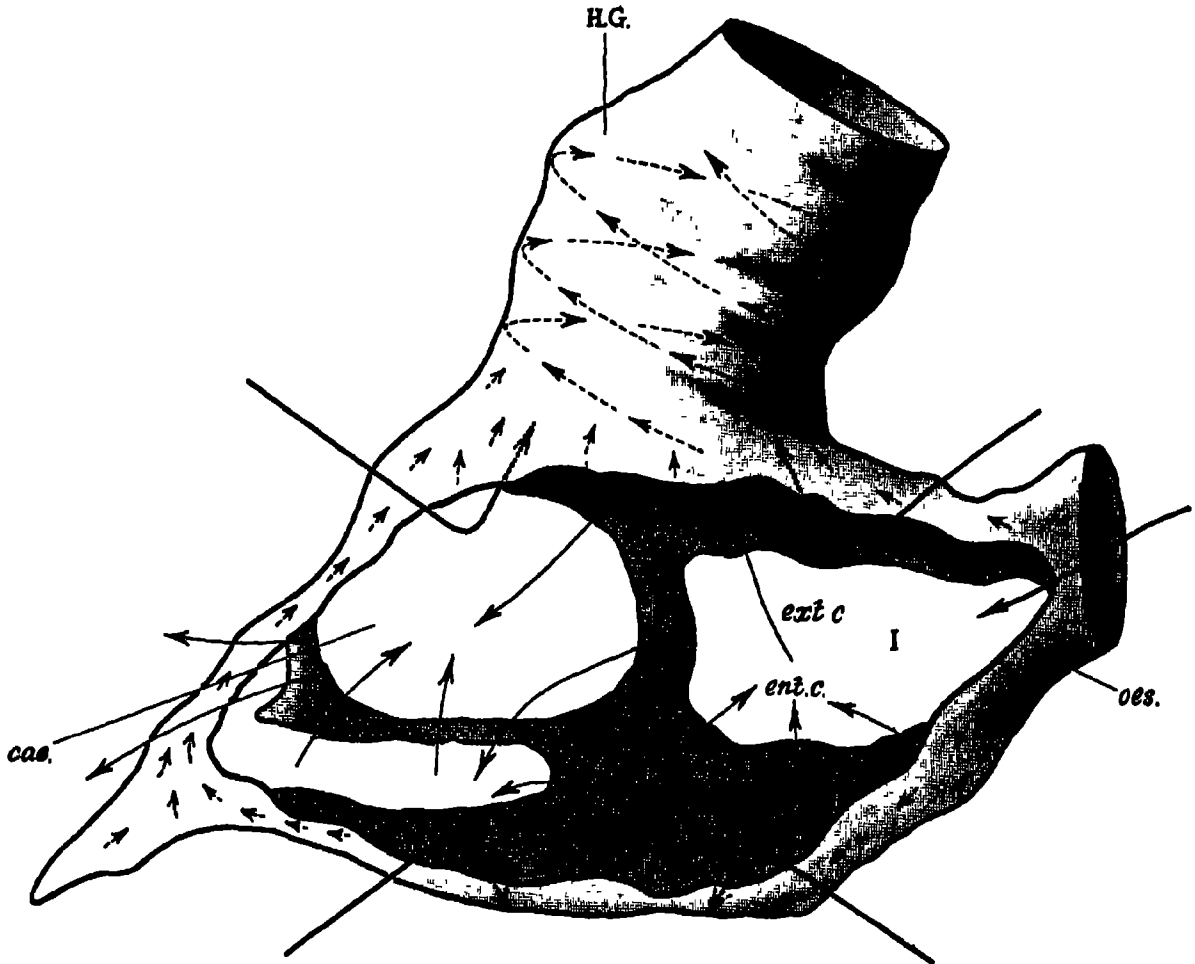


FIG. 18—Diagram showing ciliary currents of the midgut. The midgut is isolated from the caecum, and the surrounding digestive gland, and is viewed from the right side and slightly from above. The dotted arrows are seen by transparency. *cae.* caecum; *ent.c.* current entering digestive gland; *ext.c.* current leaving digestive gland; *H.G.* hindgut; *oes.* oesophagus; *I* left antero-lateral opening to digestive gland. \times about 50.

It has not proved possible to discover any system in the midgut whereby the difference in size of the openings to the digestive glands and the peculiar distribution of the cilia about these openings, could have any significance in maintaining a circulation through the tubules of the digestive gland successively, and in a definite rotation.

There is no system ensuring a perfect separation of food and faeces. In the absence of such a system the gut of *Jorunna* differs markedly from that of *Patella* (GRAHAM 1932), where there exists a valve-like thickening of the gut wall ensuring the separation of food and faecal particles.

Transference of Particles along the Hindgut—The ciliation of the hindgut was found to be exactly the same as that of the oesophagus.

Loose faecal particles are mixed with a copious secretion of mucus from the gut wall, and carried down the hindgut in the three tracts *A*, *B* and *B'*. As in the oesophagus, since *A*, *B* and *B'* are close together, the number of particles present is usually large enough to bring about coalescence of the three tracts to form a single bolus of faeces.

Movement of Particles in the Caecum (see fig. 19)—The caecum is ciliated throughout, but its small size and delicate structure, and the fact that it lies deeply embedded in the digestive gland, renders excision of the intact structure impossible. Consequently it is here that the method of studying ciliary action from sections of fixed material has proved most valuable and the following account of the ciliation has been entirely deduced by this method.

The cilia round the opening of the caecum into the midgut beat inwards (heavy arrows, fig. 19), thus drawing all particles in the vicinity of the opening into the caecum. The majority of these particles are swept on to the ridges at the back of the caecum.

The ciliation of these ridges and their complementary grooves is perfectly uniform. The cilia on the summit of each ridge beat along the main axis of the ridge and towards the head of the caecum (*h.*). These may be termed the frontal cilia. The cilia on the sides of the ridges (these may be termed lateral cilia) beat downwards into the grooves, while the cilia lining the base of the grooves (basal cilia) beat along the grooves, and again in the direction of the head of the caecum.

The upper ends of the lateral series of ridges (*lat.r.*) and grooves (*lat.g.*) are closely approximated to the mesial series (*mes.r.* and *mes.g.*), and hence, as a consideration of fig. 19 will show, all particles passing along the lateral series will eventually be taken up by the cilia of the mesial.

Thus the large particles are taken up by the frontal cilia and carried upwards on the tops of the ridges to the head of the caecum. The particles which are fine enough to pass into the grooves between the ridges, are swept down to the base of the grooves by the lateral cilia, and thence carried to the head of the caecum by the basal cilia.

Particles taken up by the cilia on the dorsal side of the opening of the caecum into the midgut are carried directly to the head of the caecum by the cilia lining the inner wall (*i.w.*). Particles passing into the opening laterally are caught up by the cilia lining the inner wall of the fundus (*f.*), and carried on to the lateral series of ridges and grooves, while particles passing in by the ventral side of the opening are carried by the cilia lining the base of the caecum on to the bottom of both series of ridges and grooves.

As a result, all particles which enter the opening from the midgut are carried up to the head of the caecum. The mass of particles which accumulates there is rotated

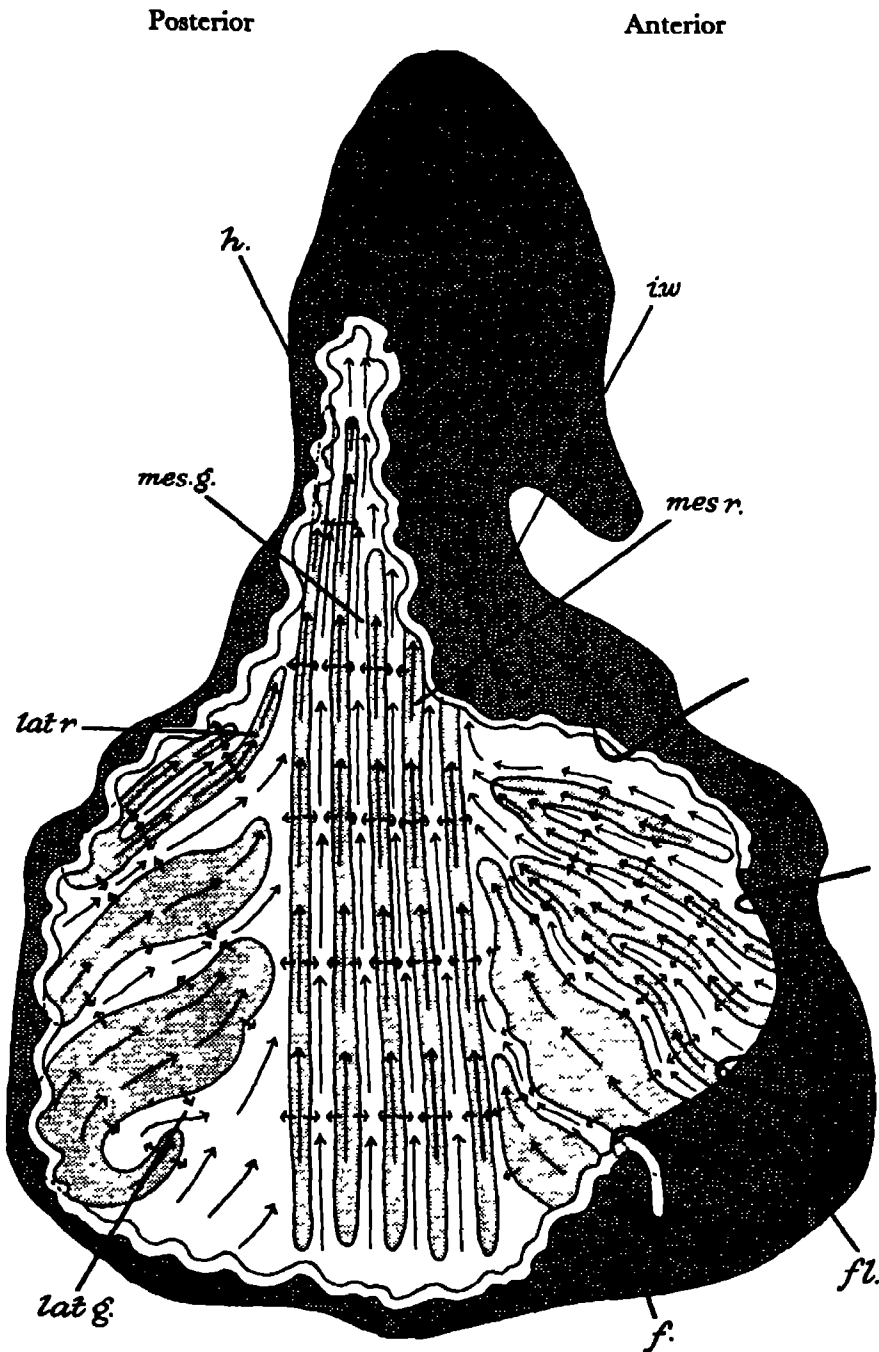


FIG. 19.—Diagram showing ciliation of the caecum. The caecum is viewed from the midgut. Part of the wall, in the vicinity of the opening to the midgut, has been cut away so as to show the course of the ridges inside. The dotted arrows are seen by transparency. *f.* fundus of caecum; *fl.* flange formed by piece of wall of midgut; *h.* head of caecum; *i.w.* inner wall; *lat.g.* lateral groove; *lat.r.* lateral ridge; *mes.g.* mesial groove; *mes.r.* mesial ridge. \times about 70.

by the action of the cilia which line the walls. The importance of this is discussed later. Evidence fully substantiating these deductions was obtained from a consideration of the peculiar distribution of particles inside the caecum.

Coarse particles are always found aggregated in small masses on the tips of the ridges, while fine particles are found within the grooves or in the act of descending into them. The only region where fine or coarse particles accumulate is in the head of the caecum, where they form a compact bolus.

Thus all particles entering the caecum must be passing up to the head along the tops of the ridges and in the grooves. All the series of sections examined gave perfectly consistent evidence for these deductions.

Movement of Particles in the Gut—II. By Muscular Contraction of the Walls

The oesophagus and hindgut of *Jorunna* lie relatively free within the body cavity, the only attachment to surrounding organs is by thin mesenteries of connective tissue. This fact has considerable significance, since it admits of the possibility of peristalsis, or some similar muscular movement of the gut wall, playing a part in the transference of particles.

The possible existence of such a mechanism is confirmed by the fact that if the oesophagus, or hindgut, be removed by dissection and examined in sea water, they undergo vigorous writhing movements due to muscular contraction of their walls. In addition, occasional waves of contraction, possibly comparable to the peristaltic contraction of the gut of vertebrates, may be observed in the excised pieces of gut. It appears that the movements are under nervous control since they may be accentuated by pricking the gut wall with some such sharp instrument as a dissecting needle, though whether these movements exhibit the characteristics of the true peristalsis of the vertebrate intestine, and whether therefore they should be termed bona fide "peristaltic movements", is a matter for further investigation. It is interesting to note that YONGE (1925*b*) has observed what he describes as "peristaltic action" in the gut of *Archidoris britannica*, and similar movements in the oesophagus of the carnivorous Septibranchs, *Poromya* and *Cuspidaria* (1928).

The Effect of Temperature on the Transference of Food along the Gut

Several specimens of *Jorunna* which had been starved for several days and which had ceased to pass faeces—thus showing that the gut was clear of food—were fed on powdered carmine, by injecting a suspension of carmine in sea water into the buccal cavity by means of a fine pipette. Care was taken not to force the injection down the gut, but only into the buccal cavity.

Groups of four specimens so treated were placed in baths of fresh sea water at known constant temperatures, and the time required for the first appearance of the

carmine-coloured faeces was noted. The average time required in each of the groups of four specimens are given below:

- Temp. 18° C.: faeces appeared 8·5 hr. after feeding;
- Temp. 21° C.: faeces appeared 7 hr. after feeding;
- Temp. 23° C.: faeces appeared 6·5 hr. after feeding;
- Temp. 25° C.: gut movement ceased, animals died.

Hence food passes most rapidly through the gut at 23° C.

The various temperatures probably affect the rate of movement of the gut wall, the rate of movement of the cilia, and the rate of digestion in the digestive gland, but to different extents.

VII—THE ENZYMES

The enzymes present were determined by dissecting out the appropriate regions of the living gut, and, after freeing them as far as possible from surrounding tissues, grinding them up in clean freshly scalded utensils, with clean dry sand and distilled water. The extract thus obtained was filtered several times, and then disinfected by the addition of a few drops of toluol to prevent bacterial action. In order to obtain a strong extract, a considerable number of animals were treated in this way, and as little water as possible was used.

The extracts were tested for proteolytic, lipolytic and amylolytic enzymes by the usual methods.* The experiments were carried out at 30° C. for 20 or 48 hr. Toluol was added to prevent bacterial changes during the period of digestion. Active extracts were obtained from the liver only. This extract contained two proteases (acting in acidic and alkaline media respectively), lipase and possibly amylase. No enzymes were found in the extracts of the buccal cavity, oesophagus, midgut or hindgut. The absence of enzymes from the midgut indicates that the liver enzymes are intracellular.

VIII—INGESTION

In order to ascertain the region of the gut where ingestion occurs, specimens were fed with substances that could easily be traced during their passage through the gut.

A number of animals were fed on neutral olive oil stained with Nile-blue sulphate, others with fish blood, and others with iron saccharate. In each case the substances were introduced into the gut from a fine pipette inserted into the buccal cavity, care

* The following substrates were used: for proteases, Congo Red fibrin, acid casein (at pH 1·2), and alkaline casein (at pH 8·5); for amylases, starch, sucrose and gum arabic; for lipase, phenol-red milk. In order to detect digestion, the casein solutions were subjected to precipitation tests (COLE 1928, pp. 250 and 260), and the incubated carbohydrate substrates were tested for reducing sugars by BENEDICT'S method.

being taken not to force the substances along the gut more than was absolutely unavoidable.

The specimens fed on fish blood and iron saccharate were fixed after known successive intervals, the former in Flemming without acetic, the latter, by YONGE's modification of the usual method after iron saccharate feeding, viz. in a mixture of equal parts of 5% ammonium sulphide in 95% alcohol, and Bouin's fluid. Sections were cut at 10 μ .

The digestive glands of specimens fed on olive oil were removed, teased out and examined in the living state. The stained olive oil was easily traceable and furthermore the process of digestion could be followed, since the neutral oil stains red in Nile-blue sulphate while fatty acids stain blue.

Sections of specimens fed on fish blood were stained in safranin and light green, after which the blood corpuscles were easily traceable owing to their avidity for safranin.

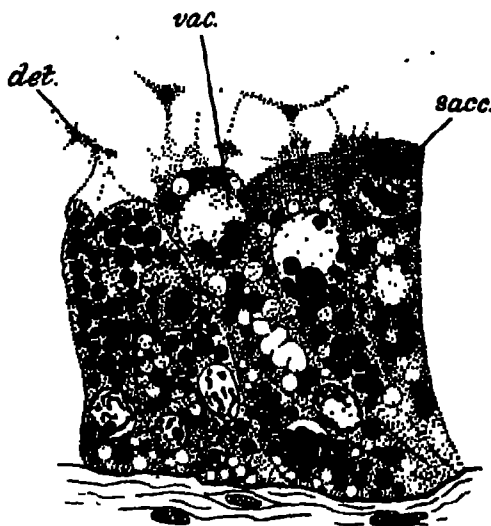


FIG. 20—Portion of transverse section of digestive gland epithelium of a specimen fixed 9 hr. after feeding with iron saccharate. Fixed as described in text. Stained in eosin. *det.* particles of detritus, possibly extruded from underlying cells; *vac.* large vacuole containing particles of iron saccharate; *sacc.* ingested particles of iron saccharate. $\times 750$.

Sections of specimens fed on iron saccharate were immersed for a few minutes in a 10% solution of potassium ferrocyanide and then in dilute hydrochloric acid. This treatment converts the iron into readily detectable Prussian blue. The sections were counter-stained in eosin.

It was found that iron saccharate and blood corpuscles were ingested exclusively by the digestive gland cells, and the phagocytes abstracted from them (fig. 21).

The appearance of the digestive gland epithelium 9 hr. after feeding with iron saccharate is shown in fig. 20. The iron saccharate appeared in the form of large well-defined masses (*sacc.*), ingested within spacious discrete vacuoles (*vac.*).

The mode of absorption of the iron, viz. in the form of large well-defined masses inside large vacuoles, is strongly indicative of intracellular digestion (YONGE 1926*a*), and differs from the true absorption described by HIRSON (1924) in the salivary glands of *Murex trunculus*, where the iron appears in a diffuse state ("diffuse stadium") until about 24 hr. after feeding. This evidence is fully supported by the mode of absorption of blood corpuscles which were seen to be ingested in large vacuoles (*vac.* fig. 23) in both liver cells and phagocytes.

In sections of a specimen fixed 8 hr. after feeding, successive stages in the process of digestion can readily be observed. Three stages are shown in figs. 22, 23 and 24. Fig. 22 shows a blood corpuscle (*cpl.*) in the process of being ingested by a phagocyte which is just flowing round it. Fig. 23 depicts a later stage. The corpuscle is enclosed in a large vacuole and is beginning to show marked signs of erosion. The envelope is becoming irregular, while the contents are being converted into oil-like droplets (*d.*). The nucleus shows obvious signs of degeneration. Fig. 24 shows the final stage, the corpuscle has been completely resolved into oil-like droplets (*dp.*), which show a marked avidity for safranin, while the cytoplasm appears blackish after the osmic fixation owing to the presence of abundant fatty substances.

Further confirmation is afforded by the presence of the digestive-gland cells and phagocytes (fig. 25) of specimens fed on olive oil stained with Nile-blue sulphate. In the digestive gland of individuals examined 5 hr. after feeding, the oil was seen to be ingested within large vacuoles and was clearly undergoing digestion as shown by the change in colour of the Nile-blue sulphate. Vacuoles stained in colours varying from the pink of the neutral oil to the blue of the fatty acids were seen.

The results of the above experiments are in complete accordance with the evidence gained by a study of the enzymes which were seen to be exclusively intracellular, and with the histological structure of the digestive gland described on p. 189, where it was shown that secreting cells are absent from the gland.

The indigestible remainder of the food is expelled from the vacuoles of the digestive gland cells and phagocytes at the free cell surface. Frequently, masses of detritus can be seen clinging to the cells as if in process of being extruded (fig. 20).

Discussion—The evidence afforded by feeding experiments indicates conclusively that digestion occurs only in the digestive gland. This is in complete accordance with the results gained from a study of the enzymes, which were found only in the digestive gland.

The absence of enzymes from the midgut (which communicates freely with the digestive gland), indicates that no extracellular enzymes are produced by the digestive gland cells. This was supported by the feeding experiments with fish blood which showed that the corpuscles underwent no erosion whilst they remained in the cavity of the midgut. Digestion was only seen to occur *inside* the phagocytes and cells lining the digestive gland.

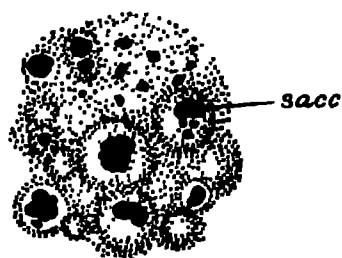


FIG. 21

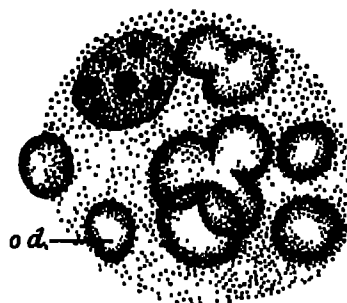


FIG. 25

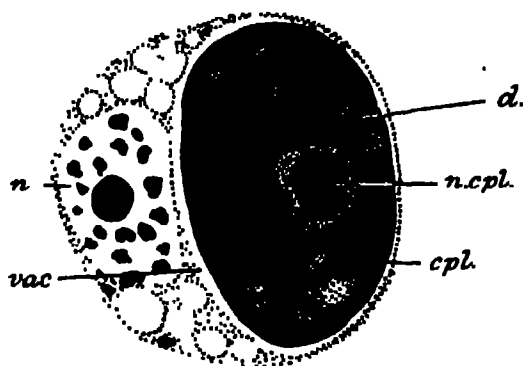


FIG. 23

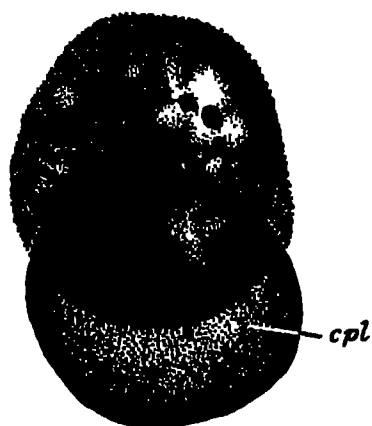


FIG. 22

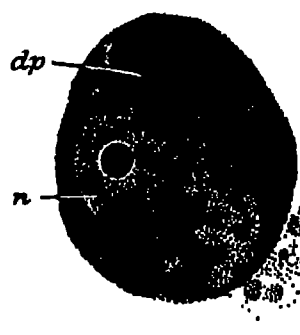


FIG. 24

FIG. 21—Phagocyte with ingested iron. From digestive gland of a specimen which had been fixed 24 hr. after feeding on iron saccharate. Fixed and stained as described in text. $\times 3000$.

FIGS. 22, 23, 24—Successive stages in the digestion of fish blood corpuscles by phagocytes, seen in a specimen fixed 8 hr. after feeding on fish blood. Fixed Flemming without acetic, stained safranin and light green. $\times 3000$.

FIG. 25—A phagocyte from the digestive gland of a specimen which had been teased up 24 hr. after feeding on olive oil. Cleared and mounted in glycerine. $\times 3000$.

cpl. blood corpuscle; *d.* oil-like droplet in corpuscle; *dp.* droplets staining in safranin, derived from digested corpuscles; *n.* nucleus of phagocyte; *n.cpl.* nucleus of blood corpuscle; *o.d.* ingested droplet of olive oil; *sacc.* ingested particles of iron; *vac.* vacuole in phagocyte.

The occurrence of intracellular digestion in the liver is in complete harmony with the results gained by several workers on Mollusca, notably VON BRUEL (1904) in *Caliphylla* and *Hermaea*, HIRSCH (1924) in *Murex*, PECZENIK (1925) in *Limnea*, GRAHAM (1932) in *Patella*, YONGE (1931) in *Doris* and Gymnosomatous pteropods, and HÖRSTADIUS (1933) in *Pleurobranchia* and *Hermaea*.

The results of the feeding experiments also show beyond all doubt that the amoeboid cells described in the previous sections as phagocytes are really such, and that their function, like that of the digestive-gland cells, is to take up particles of food from the lumen of the gut.

IX—THE FAECES

When the faeces are examined microscopically, they are seen to consist of an interlocking mass of sponge spicules and debris, the components of which are largely unrecognizable, rendered consistent by viscid secretions from the walls of the caecum and hindgut. They form brownish, slightly irregular, rod-like pellets which are slightly pliable.

Preparation of the Faeces—Sections of the head of the caecum (see fig. 26) reveal the presence of a large bolus of detritus (*b.d.*) inside the lumen. The bolus is rounded, and consists largely of compacted, unrecognizable debris, among which effete phagocytes (*p.*) and fragments of sponge spicules (*spic*) may sometimes be seen, and the whole is wrapped in an even layer of secretion (*sec.*), which stains pale blue in Mallory. Judging from the size, shape, and composition of the bolus, there can be little doubt that it is a faecal pellet.

Since the head of the caecum is the *only* region of the gut where such compacted masses of debris are constantly found, and furthermore since all stages in the formation of the faecal mass, from the early accumulation of effete particles to the final stages where a regular compact bolus is formed, may be observed in sections of the head of the caecum, there can be little doubt that it is here that the faecal pellets are prepared.

Bearing in mind the course of the ciliary currents within the caecum discussed on pp. 204–6, the process of formation of the faecal mass may be reconstructed as being thus:

Effete particles, together with mucus, are driven into the caecum, by the cilia around the opening (*o.cae.* figs. 1 and 3) from the midgut. They are then taken up by the cilia lining the walls, and carried directly to the head of the caecum, where, partly in consequence of the pressure of the continual upward current, and partly because of the pressure exerted by the walls, which in this region are provided with strengthening devices (see p. 189), and a well-developed muscular coat (*circ.m.* fig. 26), the loose particles become compacted into a bolus. Meanwhile the whole mass is surrounded by viscid secretion (*sec.*) derived from surrounding gland cells (*g.c.*), and at the

same time it is being rotated by the action of the cilia lining the wall of this region. This motion has the effect of rubbing the bolus against the walls of the caecum and thus smoothing it out in the manner of a potter's wheel, converting it into a smooth and regular faecal pellet.

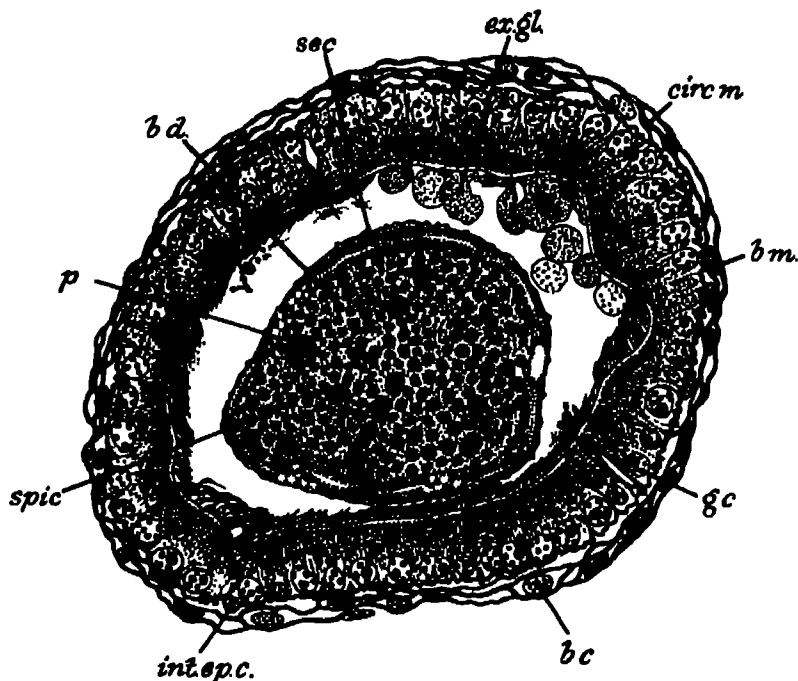


FIG. 26—Transverse section of head region of caecum, showing a faecal bolus in process of formation. From a preparation fixed in Duboscq-Brasil and stained in Mallory. *b.c.* blood cell; *b.d.* faecal bolus; *b.m.* basement membrane of epithelium; *circ.m.* circular muscle band; *ex.gl.* globule of excreta (see p. 180); *g.c.* gland cell producing viscid secretion; *int.ep.c.* structure resembling intraepithelial canal (see p. 197); *p.* phagocyte; *sec.* layer of secretion; *spic.* sponge spicule embedded in faeces. $\times 387$.

The means by which the faecal masses leave the caecum could not be determined. There is no evidence for the occurrence of a ciliary reversal in the caecum, and it therefore seems most probable that the faecal mass is forced out of the caecum and into the midgut by muscular contraction of the head region, possibly by a peristaltic-like movement, induced periodically by the pressure of faecal matter against the walls of the caecum head. The presence of a well-developed layer of circular muscles (*circ.m.*) in the wall of this region of the caecum supports this conjecture.

The faecal bolus thus ejected, would be carried away by the powerful, spirally ascending currents at the beginning of the hindgut (see p. 202).

Since the length of the faecal masses ejected by living animals was usually far in excess of that ever observed for a faecal bolus contained in the caecum, probably several such boli may become joined together during their passage down the hindgut. It is probable also that faecal masses produced in the caecum are supplemented during

their passage down the hindgut by faecal particles that have been carried directly from the midgut, the whole being bound together by mucoid secretion derived from the abundant goblet cells (see p. 195) in the hindgut epithelium.

The form of the faeces recalls that of the faecal mass of *Patella* described by GRAHAM (1932), where the faecal particles are welded into a central consistent rod surrounded by peripheral layers of cement. The similarity is correlated with a similar need on the part of both animals.

In *Patella*, which remains attached to rocks for long periods, it is important that no disintegration of faeces occurs in the vicinity of the anus, resulting in fouling of the mantle cavity (GRAHAM 1932).

In *Jorunna*, where the anus lies within the circlet of branchiae, it is equally essential that no disintegration of faeces occurs.

The fact that the mechanism for the preparation of faeces in *Jorunna* has not the complexity of that of *Patella*, where the whole of the elaborate midgut appears to be largely concerned with preparation of faeces, is possibly correlated with a different mode of life. *Jorunna* is comparatively active, and slight disintegration of the faeces would be of no consequence, since the naked gills are continually bathed by currents of clean water; and the movement of the animal will tend to shake off any loose faecal masses that may remain clinging to the gills. *Patella*, on the other hand, remains in one situation for considerable periods, and thus the chances of fouling of the mantle are considerably greater, hence the more elaborate mechanism for preparation of the faeces.

X—GENERAL DISCUSSION

It is remarkable that the alimentary canal of so specialized a mollusc as *Jorunna* has retained such a simple form. The only morphological specialization lies in the secondary detorsion that has occurred, resulting in the hindgut taking up the form of a U. Detorsion is a characteristic feature of all nudibranchs.

The radula is clearly adapted to the peculiar diet of sponge, which is soft, yet contains large numbers of spicules, in that it possesses a large number of stout, simple teeth; there are no central teeth, and there is little differentiation into laterals and marginals. Feeding on sponges necessitates taking into the gut large numbers of spicules. Since these are forcibly thrust into the oesophagus by the radula, the delicate oesophageal epithelium is rendered liable to injury. As a protection, the food is embedded in particularly large quantities of mucus secreted by the extremely abundant goblet cells in the oesophageal wall. Possibly, the process of coating the faeces with viscid secretion (see above) is to some extent a similarly protective measure.

The presence in the gut of large numbers of phagocytes, which have been shown to ingest food particles, indicates the importance of intracellular digestion in *Jorunna*. Their occurrence is noteworthy since YONGE (1926*a*) states that phagocytes are absent in gastropods.

Since the openings from the midgut into the digestive gland are large, the phagocytes cannot form a special provision for ingesting larger particles than those which can enter the digestive gland, as YONGE (1926*a*, 1931) suggests is the case in the lamellibranchs. Moreover, feeding experiments showed that all particles, whether large or small, were ingested indiscriminately by both phagocytes and digestive gland cells. The ultimate fate of the phagocytes was not determined. If they wander back to rejoin the digestive gland epithelium, and thus transfer the products of digestion of the food particles taken up, to the digestive gland cells, then it may be suggested that the significance of the phagocytes lies merely in the fact that they form a convenient means of extending the ingestive surface of the digestive gland into the lumen of the midgut. The phagocytes are not comparable with those described by YONGE and other workers in the gut of lamellibranchs, since they are confined to the lumen of the digestive gland and midgut, and not in any way connected with the blood system, whereas the phagocytes of lamellibranchs wander freely in and out of the lumen of the gut and through all the tissues, and are essentially blood cells.

The investigation of the enzymes, though regrettably incomplete, is sufficient to show that in accordance with the specialized diet, the enzymes of *Jorunna* are different from those of typical herbivorous gastropods, such as *Crepidula* or *Aplysia*, on one hand, and from those of typically carnivorous forms, such as *Murex* or *Natica*, on the other.

As would be expected in a carnivore, a powerful protease is present, though it is intracellular. In this latter particular *Jorunna* resembles the lamellibranchs, including the herbivorous forms such as *Mya* (YONGE 1923), *Ostrea* (YONGE 1926*b*), *Ensis* (GRAHAM 1931), as well as the carnivorous septibranchs *Poromya* and *Cuspidaria* (YONGE 1928), and the herbivorous gastropods which possess a crystalline style, such as *Pterocera* (YONGE 1931 and 1932*a*).

It is remarkable that the enzymes are exclusively intracellular, and correlated with this is the existence of large openings between the midgut and digestive gland, which allow large particles to be ingested directly by the digestive gland cells.

The degree of adaptation of the enzymes to the diet recalls that of the highly specialized lamellibranchs *Poromya* and *Cuspidaria*, which is described by YONGE (1928). In both cases the animals feed on large pieces of animal tissue, which they digest with the aid of a powerful intracellular protease.

In the presence of a powerful protease these animals resemble the typical carnivorous gastropods, but it is an important distinction that the protease of *Jorunna* like that of *Poromya* and *Cuspidaria* is intracellular, whereas that of the carnivorous gastropods is extracellular. The difference is possibly correlated with a different phylogenetic history.

The resemblance is probably due to convergence, both forms adapting themselves to a diet of meat, but whereas the carnivorous gastropods apparently had no immediate herbivorous ancestors, and therefore no limitations were placed on their

diet, *Poromya* and *Cuspidaria*, although able to strengthen the weak protease of their herbivorous ancestors, were unable to evolve an *extracellular*, form an *intracellular*, enzyme (YONGE 1928). Possibly, the occurrence of an intracellular protease in *Jorunna* admits of a similar explanation.

Thus the digestive processes of *Jorunna*, like those of *Poromya* and *Cuspidaria*, may possibly be an adaptation of the predominantly carbohydrate digesting processes of a herbivorous ancestor for a carnivorous diet.

A similar change of diet in two groups with Molluscan organization would be expected to have similar physiological effects, and this is exactly what we find, the protease in each case being strengthened.

It is interesting to note the effect of a similar diet on groups of animals with different organization. *Sabella* among the annelids and a tunicate such as *Ciona* are ciliary feeders, feeding on the same fine suspensions of particles as the lamellibranchs, and yet the enzymes of the two former differ distinctly from those of the latter in being entirely *extracellular* (NICOL 1930; BERRIL 1929; and YONGE 1925a). Hence we must conclude that similarity of food, and similarity of the means whereby it is obtained, are not sufficient to bring about similar digestive processes. Obviously some other factor must be involved, and very probably this factor lies in the difference of organization of the three groups Polychaeta, Tunicata and Mollusca.

XI—SUMMARY

1—The anatomy and histology of the alimentary canal of the sponge-eating nudibranch mollusc *Jorunna tomentosa* are described.

2—The mouth leads into the buccal cavity, which is provided with three pairs of lips, and houses the odontophore. It is followed by a simple oesophagus which expands to form the sac-like midgut.

3—Into the midgut open the caecum and complex racemose digestive gland.

4—The U-shaped hindgut arises from the dorsal aspect of the midgut. Its peculiar shape is due to the secondary detorsion so characteristic of nudibranchs.

5—In accordance with its tritulating function, the buccal cavity is lined by a hard cuticular layer, the staining properties and mode of origin of which are discussed.

6—The oesophagus, midgut and hindgut, are lined by ciliated and mucus cells, the relative proportions of which are to some extent correlated with the particular function of each region. In addition, the oesophageal epithelium houses a few gland cells of unknown function.

7—The caecum is lined by ciliated cells, and small gland cells which produce a secretion aiding the cementing of effete particles into a compact faecal bolus. Certain of the ciliated cells cut off their outer ends as rounded bodies which lie free in the caecum. The significance of this is discussed.

8—Lining the digestive gland are large cells which ingest food particles. Their free ends may occasionally bear cilia or abstrict phagocytes.

9—Numerous phagocytes, the mode of origin of which is described, lie inside the lumen of the midgut, digestive gland and caecum.

10—Wandering cells are common in the lumen, epithelium and submucosa of the gut.

11—The submucosa consists of connective tissue within which are large mucus cells, and longitudinal, circular and oblique muscle fibres.

12—Feeding, and the action of the radula are described.

13—Food is transferred along the gut by means of cilia, possibly aided by muscular contraction of the gut wall. The complete elucidation of ciliary currents was only rendered possible by the use of an entirely new method.

14—Particles are carried down the oesophagus in three main tracts. On entering the midgut, they are taken up by the cilia lining the openings of the digestive gland, and circulated through the tubules. Effete particles are returned to the midgut and either taken directly into the hindgut, or wafted into the caecum, where they are compacted into a faecal bolus. There is no food-sorting mechanism.

15—The effect of temperature on the passage of food along the gut is described.

16—Enzymes are produced only by the digestive gland. They are exclusively intracellular, and digest fats and proteins. It is uncertain whether any carbohydrate is digested.

17—Feeding experiments indicate that the phagocytes and digestive gland cells perform the function of ingestion.

18—The formation of compact faeces is described, and their significance is discussed.

19—The considerable morphological and physiological adaptation of the gut to the peculiar diet of sponge is discussed, and the results of the investigation are viewed in the light of some recent work on the alimentary systems of other Molluscs and phyla. The peculiar nature of the enzymes is regarded as a possible indication of an herbivorous ancestor.

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V—THE DEVELOPMENT OF *HALIOTIS TUBERCULATA*, WITH SPECIAL REFERENCE TO ORGANOGENESIS DURING TORSION

By DORIS R. CROFTS, M.Sc.

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[Plates 21–27]

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INTRODUCTION

Investigation of the development of *Haliotis tuberculata*, LINNAEUS, was undertaken as a natural sequel to an examination of the bionomics and anatomy of *Haliotis* (CROFTS 1929, p. 159). It is an attempt to elucidate the organogenesis during the changing larval habits, in the hope of adding to the scanty ontogenetical evidence available towards solving the problems of gastropod evolution.

Excellent accounts of the embryonic phase, including patiently worked out details of the cleavage and of gastrulation, have been published for *Crepidula* (CONKLIN 1897), *Patella* (PATTEN 1886; WILSON 1904; and SMITH 1935), *Trochus* (ROBERT 1902), *Physa* (WIERZEJSKI 1905), *Dentalium* (WILSON 1904). The only comprehensive accounts of gastropod larval development already published are of *Paludina* (ERLANGER 1891; TONNIGES 1896; DRUMMOND 1902; ANDERSEN 1924) and of *Patella* (PATTEN 1886; SMITH 1935). The accounts of *Paludina* elucidate the details of development of the coelom and its derivatives. The viviparous habit and consequent loss of free larval life in *Paludina*, however, make it a very unsuitable example of gastropod development. Evolutionary stages are more likely to be traced from the development of less specialized gastropods, an adequate selection of whose free-living larval stages can be obtained. The larval development of *Patella*, *Acmaea*, *Trochus* and *Haliotis* has been described, in varying completeness for the different genera. PATTEN's work on *Patella coerulea* (1886) gives a description only of the beginning of organogenesis. After a lapse of fifty years a more detailed account of the development of *P. vulgata* has been given by SMITH (1935). This work gives a new interpretation of the development of the mesoderm, of the muscles and of the nervous system. The dorsal retractor muscle of the larva, which PATTEN indicated for *P. coerulea*, is shown in *P. vulgata* to be placed asymmetrically and torsion takes place "under the action" of this muscle.

ROBERT (1902) gives an excellent account of the development of *Trochus*. His description, however, does not extend to the development of internal organs. In his account of the development of *Acmaea* and *Haliotis*, BOUTAN (1899) also confines his attention to external characters and habits, but the age of the stages described for *Haliotis* was unknown and he was unable to determine at what age the swimming larvae begin to creep. BOUTAN's claim to have observed complete 180° torsion in the rapid twist of the pelagic *Acmaea* and *Haliotis* larvae, upon which various theories have been based, is not confirmed by the account given in the following pages, which, unlike his account, is based on the examination of serial sections and reconstructions. BOUTAN's drawings and descriptions of the external features of the post-veliger creeping stage are of interest. STEPHENSON (1924), when undertaking a short economic investigation of *Haliotis*, kept larvae alive only for 62 hr. after fertilization.

MURAYAMA (1935) gives a superficial account of the development of the Japanese abalone, *Haliotis gigantea*, which adds but little to BOUTAN's description for *H. tuberculata*.

Additional information concerning the details of organogenesis and metamorphosis of primitive gastropods is still highly desirable before the various theoretical views concerning the peculiarities of this group can be either substantiated or modified.

The Haliotidae (FLEMMING) is a somewhat more primitive family of the Archaeogastropoda (THIELE) than the Patellidae (GUILDING). In addition to the retention of paired kidneys and osphradia, *Haliotis* possesses paired auricles, hypobranchial glands and ctenidia; the rectum, as in lamellibranchs, is ensheathed by the heart. The following description concerns mainly the post-embryonic development of the Sarnian species

tuberculata, in which the full complement of these adult paired characters is acquired only after 2 months of development.

My thanks are due to M. CHARLES PEREZ and M. GEORGES TEISSIER of the Biological Station at Roscoff and to Dr. PHILIPPA ESDAILE of King's College of Household and Social Science, London University, for the provision of excellent facilities for the investigation.

To the Royal Society of London I am indebted for a Government Grant for the purchase of a high-power binocular microscope for use during the investigation.

METHODS

Haliotis eggs were fertilized artificially at the Roscoff Biological Station throughout the summer of 1929 (p. 223).

Pelagic life lasted less than 2 days, so that the plunger-jar method for rearing larvae (ALLEN and NELSON 1910) proved less useful than was anticipated. Feeding began only in the benthic veligers, and they were so minute that *Nitzschia* was too large for food until the late veliger stage was reached. A mixed culture containing *Pleurococcus*, started from a culture supplied by the Plymouth Laboratory, was useful for the early veligers. The post-veligers were supplied with small stones bearing young stages of various red sea-weeds.

A few post-veligers lived fairly successfully in bowls with aerated and circulating sea water. Although stages up to a fortnight old were sometimes healthy, it was more difficult to rear the larvae through the post-veliger metamorphosis. Specimens of five different post-veliger stages were preserved for section cutting, including a solitary specimen, which had remained healthy for 2 months. The shell was then 2 mm. long and the final metamorphosis had taken place. Several minute specimens were collected from the rocks off Sark, and the smallest of these was completely metamorphosed and was the same size as the artificially reared specimen.

SMITH also found great mortality at the beginning of metamorphosis in *Patella vulgata* (1935). He was able to rear a few specimens as far as the first stage of metamorphosis and to observe the complete process only in one specimen. In *Haliotis*, as in *Patella*, mortality appeared to be due to feeding difficulties, sudden changes of temperature, and sometimes to the presence of pathogenic Protozoa.

As BOUTAN remarked, the larvae are extremely sensitive. Observation therefore involved considerable time and patience and, except in the very early stages, it seemed impossible to fix the larvae without contraction. Various methods of narcotizing were used, but they proved unreliable. LO BIANCO's method for extending molluscs was sometimes successful for older stages, but it was useless for the early days of development because it involved leaving the larvae in 1% absolute alcohol in sea water for at least a day. A trace of magnesium chloride was more generally used.

Segmentation stages were followed in living specimens. These and the gastrulation process were observed after treatment according to WILSON's method for *Patella* (1904). The specimens were preserved in a few drops of acetic acid in a watch-glass of sea water. Glycerine was added gradually until the specimens were quite transparent. The non-permanence of these preparations is the only disadvantage. Permanent preparations were made after placing the specimens for 3 min. in Perenyi's fluid or in Kleinenberg's picro-sulphuric acid mixture. Some of these were stained in haemalum, but the best results were obtained by staining in undiluted acetic-acid alum-carmin, used by SOUTHWELL (1930) for Cestoda. They were left for 4 hr. in this stain, then rinsed and taken up to 90% alcohol, after which they were mounted in Gurr's euparal, which has a suitable refractive index. The method used by WIERZEJSKI (1905) for *Physa* was also valuable for gastrulation stages. Complete dehydration was followed by clearing in clove oil and mounting in a fluid mixture of Canada balsam and clove oil.

At first difficulty was found with the orientation and sectioning because of the minuteness of the larvae, the earliest veligers being approximately 130μ long. The best series of sections were obtained after fixation in either Bouin's fluid or in corrosive sublimate with acetic acid; both were used hot. The larvae were tinged with eosin to facilitate orientation. The celloidin-paraffin method of embedding was employed to minimize contraction and, to aid orientation, the celloidin block was made transparent in cedar oil. Occasionally it was found difficult to obtain a complete series of sections by this method, and subsequently simple paraffin embedding proved to give quite satisfactory results. Orientation was performed, after the manner suggested by Professor GRAHAM CANNON, in molten wax under a high-power binocular dissecting microscope. Sections were cut at 6μ , except with the gastrulae and trochophores, for which a thickness of $9-12\mu$ was employed. Transverse sections of larvae, starting from the posterior end, proved to be the most useful, because it was essential to know the exact spatial relations of the pallial cavity in connexion with the torsion phases.

The first sets of serial sections were stained with Weigert's iron haematoxylin or borax-carmin with picro-indigo carmin. Excellent results were obtained later with cotton red-aniline blue (gossypimine and aniline picrate).^{*} For recommendation of this stain thanks are due to Miss S. LOCKYER and to Mr. S. GARSIDE. The best results were obtained after leaving the sections for 40 min. in gossypimine. They were then rinsed in distilled water and placed for 2 min. in aniline picrate, after which they were again rinsed and rapidly dehydrated so that the gossypimine remained only in chromatin and food vacuoles. This double staining facilitated the discrimination of the developing nervous system.

Reconstructions were found to be essential to an understanding of the serial sections. Twelve reconstructions of various stages, magnified approximately 900 diameters, were made from plywood of carefully selected thickness. For the accurate fret-sawing, from the author's camera lucida drawings of serial sections, indebtedness is acknow-

^{*} The formulae for this stain were first published by FLATTERS (1905).

ledged to Mr. N. DAVEY. Models held together by veneer sprigs were studied. The sections were then separated and the important muscles, the nervous system and parts of the digestive system were cut out and reconstructed separately.

AGE AT MATURITY AND METHOD OF SPAWNING

Spawning takes place probably only in specimens which have attained 5 cm. in length and are about 3 years old. STEPHENSON (1924) describes the spawning of a captured specimen, and CROFTS (1929) gives an account of spawning in the sea. The spawning period undoubtedly extends from June to September, but it probably continues into late autumn. The latter would account for the discovery in the Channel Islands of specimens only a few millimetres long in March and April (CROFTS). The present account shows that the post-larval metamorphosis is complete at the end of about 2 months of development.

Haliotis tuberculata is dioecious. MURAYAMA (1935) states that *H. gigantea* is also normally dioecious, but he describes one hermaphrodite specimen. My examination of large numbers of *H. tuberculata* of all sizes in the Channel Islands did not lead to the discovery of a hermaphrodite specimen. Among 280 specimens of marketable size the sexes were almost equally distributed, so that it seems unlikely that *H. tuberculata* is a protandrous hermaphrodite.

In both sexes the genital products escape via the cavity of the definitive right renal organ through the shell perforations. In the late stage of spawning some of the genital products escape from under the shell margin. In the male the spermatozoa are emitted from the shell holes in puffs like white clouds, but in the female the grey-green ova are less conspicuous. The ova are puffed upwards and then sink. The egg membrane has a thin albuminous layer and there is no protective gelatinous layer, such as is present in *Trochus*. The egg membrane has a micropyle and fertilization takes place in the sea. Inclusive of the egg membrane the diameter of the egg is about 180μ .

DEVELOPMENT UP TO THE TROCHOPHORE STAGE

The method used by WILSON with *Patella coerulea* for assisting artificial fertilization by placing the ova and spermatozoa separately for an hour in sea water made slightly more alkaline by the addition of about five drops of a 5% solution of caustic soda to 500 c.c. of sea water proved to be very successful for *Haliotis*. Although fertilization was hastened, the proportion of abnormal larvae was as small as when caustic soda was not added. The fertilizations were made from specimens which had commenced to spawn in aquarium tanks. After the specimens had been placed in separate bowls, the ova were collected as they were liberated. An emulsion of spermatozoa was used in the manner described by MACBRIDE (1914) for echinoderm fertilizations.

The development of cleavage planes was not followed in detail, but it appears to follow closely that of *Trochus*, which is described in great detail by ROBERT (1902). From artificial fertilizations of *Haliotis* started at 2.30 p.m. on 22 July, four blastomeres had formed at 6.30 p.m. After the third cleavage, which is spiral, there are four very large macromeres because of the presence of much deuterooplasm. The macromeres appear to be larger than in *Patella coerulea* (WILSON 1904) and *P. vulgata* (compare endoderm cells in SMITH 1935, fig. 2*a* and *Haliotis*, fig. 39*b*). The macromeres separate off micromeres towards the animal pole to form the three ectoderm quartettes. Thus the blastula, with a very reduced blastocoele, is formed.

Gastrulation is by epibole. From optical sections of specimens cleared by WILSON's method, it appeared that this process closely resembled that described for *Patella coerulea* (WILSON 1904), for *Trochus* (ROBERT 1902), for *Littorina* (PELSENEER 1911) and for

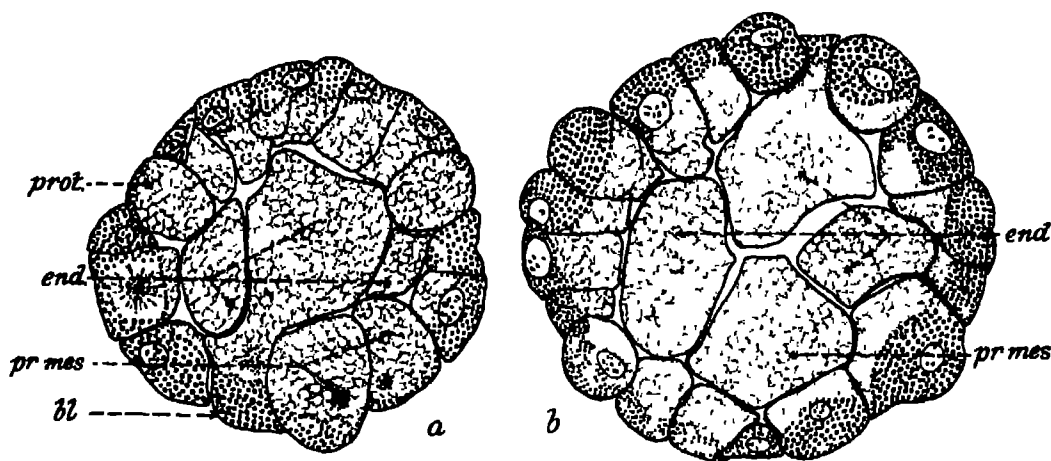


FIG. 39—*a*, parasagittal section of 7-hr. embryo, seen from left side, showing endoderm cells and two primitive mesoderm cells. *b*, transverse section of 8-hr. embryo, showing primitive mesoderm cell nearer animal pole than its partner, which is seen in fig. 40*a*. $\times 450$ linear approx.

Crepidula (CONKLIN 1897). These authors describe division of the macromere of the quadrant *D* giving a cell 4*D*, which together with the macromeres of quadrants *A*, *B* and *C* forms the endoderm, and a cell 4*d*, which is the mesoderm mother cell. Since my attention has been called to the recent careful reinvestigation of the gastrulation of *Patella* (SMITH 1935), an examination of serial sections of gastrulae of *Haliotis* has been carried out. The conclusion arrived at is that the formation of endoderm and mesoderm takes place as SMITH has shown for *Patella vulgata* and not in the manner described by CONKLIN, ROBERT and WILSON. The macromere 4*D* divides equally to form two cells which are almost spherical, in contrast with the still undivided macromeres *A*, *B* and *C*, which are irregular and much elongated in the polar direction. When first observed, the two derivatives of 4*D* are in the region of the blastopore (fig. 39*a*, *pr.mes.*). At this time large portions of each of the three other macromeres are already pressed into the obliterated segmentation cavity. The two cells formed from

4D appear to correspond with the primitive mesoderm cells of *P. vulgata* (SMITH 1935). Unfortunately a stage was not found with the division of the macromere in progress, which SMITH describes for *Patella*. During invagination of these primitive mesoderm cells, one of the pair is pushed nearer to the apical plate than the other, so that in transverse sections in that region four invaginated cells are seen which, on first examination, suggest that four macromeres take part in the formation of the endoderm (fig. 39*b*). A transverse section nearer the blastopore of the same embryo, however, shows five internal cells, two of which are primitive mesoderm cells (fig. 40*a*). It

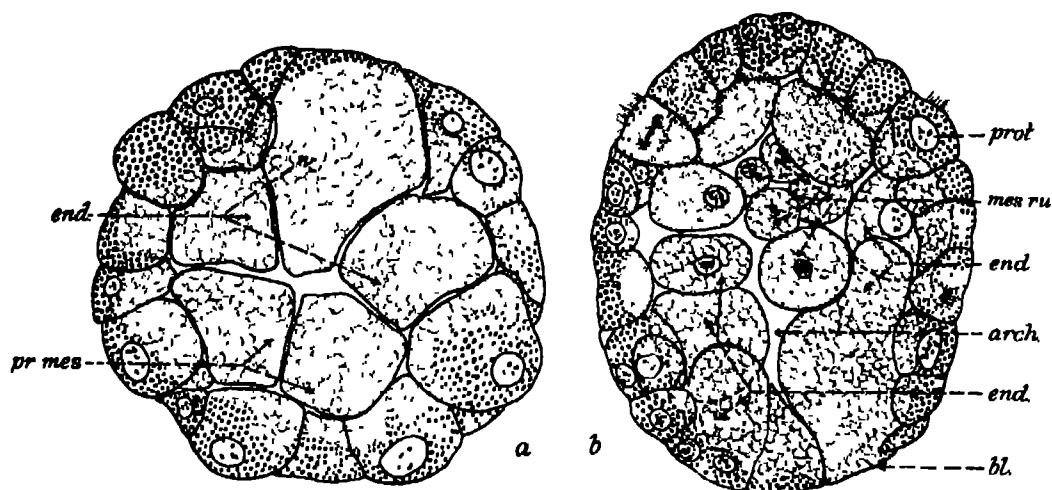


FIG. 40—*a*, transverse section of 8 hr. embryo nearer blastopore than that of fig. 39*b*, showing two primitive mesoderm cells and three endoderm cells. *b*, frontal section of 11 hr. embryo, showing division of endoderm cells and further division of mesoderm elements. $\times 450$.

seems, therefore, probable that in *Haliotis* the whole of the macromere 4D gives rise to mesoderm and three macromeres only take part in the formation of the endoderm, as in *Patella* (SMITH 1935). It may be significant that in *Trochus* that member of the pair of cells formed from 4D, which ROBERT (1902) describes as taking part in the formation of endoderm, does not divide at the time division of the endoderm cells *A*, *B* and *C* takes place.

In *Haliotis* the invaginated cells are so large that there is no room for an archenteron, and it can be discovered only after further division of these cells (fig. 40*b*).

During gastrulation there is elongation in the direction of the axis through the centre of the apical plate (fig. 40*b*). Gradual shifting of the axis of the invaginated elements takes place as the unclosed blastopore migrates towards the prototroch. The migration appears to be due to the rapid formation of mesoderm cells accompanied by rapid divisions of the ectoderm cells in the dorsal region of the embryo. ROBERT's method of indicating the migration of the axis in *Trochus* has been adopted in the figures of *Haliotis* embryos (fig. 41).

At about 8 hr. after fertilization the embryo rotates within the vitelline membrane

by movements of the cilia of the prototrochal girdle (fig. 41a). Derivation of the velum was not followed. As in *Trochus* it is monotrochal, unlike that of the trochophore of *Patella* (PATTEN 1886; SMITH 1935). *Haliotis* also differs from *Patella* in the absence of apical cilia and telotroch in the trochophore. The refractive cells and the fine cilia of the embryo cap of *Patella* (PATTEN 1886; SMITH 1935) are also lacking in *Haliotis*.

The larvae are freed at times varying from 8 to 13 hr. after fertilization when they are about 130μ long (fig. 41b). They have the trochophore characters, and the rudiments of the molluscan characters have scarcely begun to appear. According to STEPHENSON (1924) many *Haliotis* trochophores left the egg membrane and were swimming actively at 44–48 hr.; at 60 hr. "there was little difference in general form". Since STEPHENSON was unfortunate in the lack of suitable facilities, it seems probable that his larvae were arrested in development. *Patella* trochophores are freed at about 24 hr. after fertilization (PATTEN 1886). ROBERT (1902) states that *Trochus magus* larvae were freed at about 14 hr., but in other species of *Trochus* they were not freed until a much more advanced stage of development.

The primitive mesoderm cells have given rise, at the time the trochophore is freed, to ten mesoderm cells situated at the opposite end of the larva to the apical plate. There are no mesoderm pouches such as are described for *Paludina* (ERLANGER 1894). The mesoderm cells of *Haliotis* spread on either side of the primitive gut and form the mesoderm bands, which are symmetrical in arrangement only in the anterior region (fig. 23, Plate 25).

EARLY VELIGER PRIOR TO TORSION

Bionomics of the Early Veliger

This phase begins at about 9 hr. and ends not earlier than 27 hr. after fertilization.

The larvae are pelagic up to about 60 hr. after fertilization. The freed post-trochophores are positively phototropic and swim near the surface of the water with the velum directed upwards and the shell below. As in other gastropod larvae, the velum is the only swimming organ. Rotation about the axis in the field of the velum is produced by the lashing of the long cilia. It appears to be usually counter-clockwise, when the veliger is regarded from the posterior end (fig. 2, Plate 21). Occasionally the direction of rotation is reversed and now and then, especially when the water is disturbed, the cilia stop lashing and converge anteriorly over the centre of the velar field (fig. 1, Plate 21). This causes the larvae to sink to the bottom of the water, where they rest motionless for a time and then resume their rapid revolutions. Feeding has not yet begun.

Beginning of Organ Formation

The velum of the *Haliotis* veliger retains the simple character of the pre-oral ciliated ring or prototroch of the trochophore, as in *Patella* (PATTEN 1886) and *Trochus* (ROBERT

1902). When the ciliated cells have become arranged in a circle, there are sixteen cells cut off from the rest of the body by a constriction (fig. 41*b*). The velum gets very little larger, increasing to twenty-four cells only in the older veliger (figs. 1-6, Plate 21).

The blastopore has now closed and, when first the invagination for the shell gland rudiment appears, there is a groove indicating the direction of migration of the blastopore towards the velum from its original position on the axis of the velar field

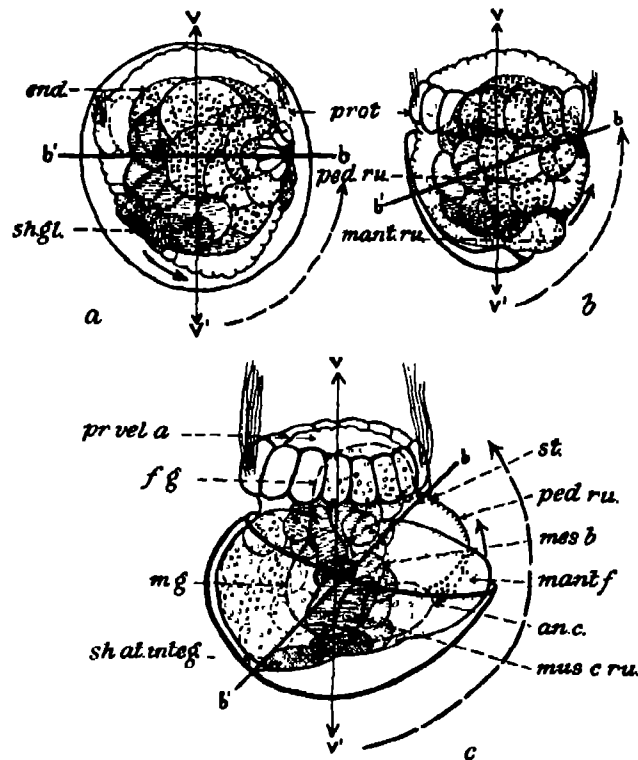


FIG. 41—Late embryo and early veligers seen from right side, drawn from whole mounts and models. $\times 170$. *a*, 11 hr., immediately before larva freed, showing rudiment of shell gland and stomodaeum in region of closed blastopore. *b*, 16 hr., showing shell rudiment and “ano-pedal flexure” begun. *c*, 19 hr., showing further stage in development of shell and flexure of primitive gut. Mesoderm cross-hatched, endoderm with small circles; *bb'* indicates axis of invaginated elements; *st.* stomodaeum; *vv'* indicates axis in field of velum.

(fig. 41*a*). This migration is completed in *Haliotis* before the proctodaeal and stomodaeal invaginations are formed. The stomodaeal invagination is well developed by 17 hr. after fertilization, in the region where the blastopore has closed just posterior to the velum (fig. 41*b* and *c*). In veligers not younger than 27 hr. old, in which 90° of torsion has been accomplished, the mouth has developed from this invagination and the foregut communicates with the endodermal part of the digestive system.

At the time the larvae are freed the foot originates as a median swelling below the united ventral lips of the blastopore.

As in *Patella vulgata* (SMITH 1935), *Trochus* (ROBERT 1902), *Crepidula* (CONKLIN 1897) and *Ischnochiton* (HEATH 1899), the pedal protuberance begins only after fusion of the blastopore lips so that the foot rudiment is never bilobed as PATTEN (1886) described it for *Patella coerulea*, although at 15 hr. it has a slight anterior groove in *Haliotis*. SMITH points out how easy it is in *Patella vulgata* to mistake enlargements due to growth of the two mesoderm bands for two pedal rudiments.

The shell gland can be recognized in *Haliotis* at about 10 hr. It is an invagination giving a slight depression on the dorsal side of the body, and it is slightly to the right side (fig. 41a).

Eversion of the shell gland is due to rapid multiplication of the cells of the gland combined with the enlargement of the primitive midgut and mesoderm bands. It occurs at 14 hr. after fertilization, or sometimes a little later, and at about 15 hr. a delicate shell of watch-glass shape has been secreted (fig. 41b). At about 18 hr. the shell gland rudiment has spread over most of the dorsal region of the body, the shell has increased round its margin into a deep saucer shape, tilted somewhat towards the right side. The body is now contracted away from the central part of the shell. This space may contain air, and in other veligers it has been thought to serve a hydrostatic purpose (fig. 41c). The shell is attached to the body at its periphery, and rapid multiplication of cells of the mantle margin, particularly at the right ventral edge of the shell, produces a thickening which is the mantle fold. For several days the shell is of transparent conchiolin and without calcareous spicules.

Ano-pedal Flexure and Subsequent Changes

Ano-pedal flexure occurs, as in all gastropod larvae, at a stage prior to torsion and, as AMAUDRUT (1898) and ROBERT (1902) point out, it must be considered as a process distinct from torsion. It involves curvature of the digestive system into a U shape so that the anus, instead of pointing posteriorly as in Amphineura and Annelida, travels round in a ventral direction; this is a result of the enlarging visceral hump, which becomes dome-shaped (fig. 41). At the completion of ano-pedal flexure, the rectum points anteriorly and lies ventrally to the stomodaeum, with the enlarging pedal mass between. In *Haliotis*, the flexure begins before the proctodaeum has formed and is complete before the stomodaeal invagination has established its connexion with the endodermal elements, which change their orientation and form the midgut, consisting of cells filled with yolk (fig. 42).

The mantle cavity originates as a result of the enlargement of the visceral mass, which causes the mantle fold, in which cells are multiplying very rapidly, to spread and encroach over the foot (fig. 41c). A gulley, which will become the pallial cavity, is thus formed between the foot and the mantle fold. In *Haliotis*, it is not at first quite in the mid-ventral position as ROBERT describes for *Trochus* and PATTEN (1886) and SMITH (1935) for *Patella*. Nor are there separate right and left mantle cavity rudiments behind the prototroch, such as DRUMMOND (1902) describes for *Paludina* and SMITH

(1935) for *Patella*. The mantle fold is first developed on the ventral right side of the body (fig. 42*b*). This may be a result of the asymmetry at the gastrula stage, when there is slight swelling on the right side in *Haliotis*, as is also described by ROBERT for *Trochus*. The mantle fold spreads over the pedal mass from its ventral and right sides (figs. 41*c* and 42). In one specimen sectioned and modelled 19 hr. after fertilization, the left side of the foot is still not covered at all by the mantle fold, but a few hours later the margin of the mantle fold has spread to the left side and most of the foot is now enveloped (fig. 43*a*).

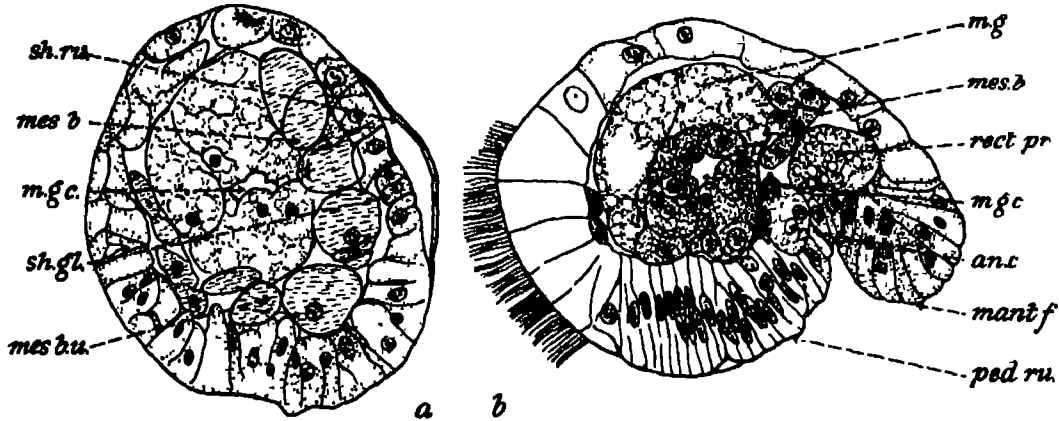


FIG. 42—Transverse sections of early veligers. $\times 450$. *a*, 17 hr., passing through shell gland and shell rudiment on right side, showing union of mesoderm bands ventrally and larger mesoderm band on right side. *b*, 19 hr., passing through mantle fold, anal cell and pedal rudiment; mesoderm cells of right band pass between midgut and primitive rectum.

At 29 hr. transverse sections show that the deepest part of the pallial cavity is mid-ventral (fig. 46*A* and fig. 31, Plate 26). The anal cell is seen on the mantle side of this region (fig. 42*b*), but it is less obvious than in *Patella* (SMITH 1935). There is scarcely any invagination when the proctodaeum forms after the first half of torsion has taken place (fig. 43).

Early in the second day the shell has changed considerably in shape; it has a wide aperture, due to rapid additions from the ventral parts of the mantle fold, and it is drawn in dorsally following the constriction between the visceral enlargement and the velum (fig. 1, Plate 21). Thus a nautiloid form with typical exogastric curvature is begun. There is also much elongation in the direction of the axis of the velum. This is due to increase in length of the visceral dome as the digestive system becomes more developed (fig. 43).

The digestive gland rudiment is a massive left dorsal swelling at about 27 hr. after fertilization (fig. 25, Plate 25), but in *Paludina*, DRUMMOND (1902) describes it as a left ventral diverticulum. The small larval stomach is lined by cubical cells which have extended to meet the invaginated ectoderm cells of the stomodaeum; the cells of the short primitive rectum are considerably larger than those of the larval stomach (figs.

25 and 27, Plate 25). Although, according to DRUMMOND (1902) for *Paludina* and PATTEN (1886) and SMITH (1935) for *Patella*, a radular sac rudiment is present at this stage, there is no sign of it in *Haliotis* until sometime after the first phase of torsion (compare fig. 30, Plate 26 with fig. 44).

The mesoderm mother cells give rise anteriorly to the mesoderm bands while ano-pedal flexure is being accomplished (fig. 41c; fig. 23, Plate 25). At 17 hr. the right mesoderm band is larger than the left (fig. 42a). Some of the more anterior cells group themselves around the dorsal and lateral parts of the ectodermal foregut, as in *Patella coerulea* (PATTEN 1886). At this time mesoderm cells of the left band have migrated into the developing foot and others of the right mesoderm band have passed into the foot and the mantle fold, which it has been shown originates on the ventral right side (fig. 42b). The two mesoderm bands unite ventrally under the rectum and between the rectum and the larval stomach at the posterior end of the mantle fold, as in *Patella* (SMITH 1935). These bands are well developed in veligers 27 hr. old. In the middle region of these larvae they are still solid bands, placed symmetrically on either side of the foregut, but their anterior ends are broken up into irregular spindle-shaped mesenchyme cells, which will develop into muscle cells and vascular tissue of the head and foot (figs. 29, 30 and 31, Plate 26).

The velum retractor muscle cells develop from the dorsal region of the right mesoderm band, which is larger than the left (fig. 42a). In *Patella* they arise from the anterior ends of both mesoderm bands (PATTEN 1886; SMITH 1935).

In *Haliotis* elongated cells can be first recognized in larvae 19 hr. old (fig. 41). Three or four of these large spindle-shaped cells are seen in sections on the right-hand side of the digestive system, posterior to the mantle cavity, and are attached posteriorly to the part of the integument which is fixed to the shell (fig. 24, Plate 25). They do not appear to be efficient contractile cells until some hours later, when the shell has become large enough to accommodate the body of the larva. In the oldest pre-torsional veligers there are six muscle cells extending the whole length of the body posterior to the velum (fig. 27, Plate 25 and fig. 43). These cells are extremely long, and are inserted anteriorly round the foregut and stomach and to the posterior part of the velum in bilaterally symmetrical fashion. They curve round the left dorsal digestive gland enlargement and pass along its right side into the integument of the right ventral part of the visceral hump, which is attached near the central part of the shell. The arrangement of these muscles is shown in figs. 15 and 15a, Plate 23; fig. 25, Plate 25. These muscle cells have very large nuclei and minute vacuoles. By the time larval torsion takes place, beginning at an age varying from 27 to 35 hr. old, this right retractor muscle is well developed, but the larvae cannot be withdrawn into the shell completely until about 40 hr. after fertilization.

Early in the second day there are invariably two spindle-shaped cells derived from the left mesoderm band. They are about one-third the length of the muscle cells of the right side and have no shell attachment. The two ectoderm cells, which make a

second posterior attachment of the visceral hump with the shell, have no muscular connexion (fig. 3, Plate 21). The two elongated mesenchyme cells are shown in transverse section in fig. 25, Plate 25. They are always in a cramped position because the bulky primitive digestive gland leans to the left side. This may be the reason why they are arrested in development for about 5 days, after which they develop into the columellar muscle of the plantigrade larva (figs. 44*a* and 46; fig. 11, Plate 22). It is, therefore, probable that the ancestors of *Haliotis* possessed paired larval retractor muscles. Although the right side retractor muscle of *Haliotis* appears to correspond remarkably closely with that of *Patella vuigata* (compare SMITH 1935, fig. 11 with *Haliotis*, fig. 1, Plate 21 and fig. 15, Plate 23), it is not possible to agree with SMITH's deductions. He assumes that this muscle was originally a median one "which has become diverted to one side by the pressure of the large yolky endoderm mass". Because an incipient representative of a left muscle is always present in *Haliotis*, it is unsuitable to adopt SMITH's term "dorsal shell retractor muscle" for the right velum retractor muscle of *Haliotis*.

The nervous system originates immediately before torsion begins, at an age varying from 27 to 30 hr. after fertilization, with the rudiments of the cerebral ganglia. They develop similarly to those of *Patella* (SMITH 1935); but, as in *Acanthochiton* (HAMMERSTEN and RUNNSTROM 1925), the rudiments are not sunken in pits of the embryo cap. Lateral thickenings of the ectoderm of the pre-velar area are readily seen in sections (fig. 31, Plate 26). The nuclei of many of these cells are undergoing mitosis; the cells gradually become rounded nerve cells. These are sunken although not completely delaminated from the ectoderm until after the first half of torsion is completed, when they form the strap-shaped cerebral ganglion band (fig. 32, Plate 26). Slightly later, paired antero-lateral thickenings of the pedal ectoderm sink into the foot and constitute the rudiments of the fused pedal ganglia. The remainder of the nervous system develops after the first torsion phase (p. 248).

Topography of the Veliger immediately before Torsion

The operculum rudiment is secreted just before torsion begins. It arises on the posterior pedal ectoderm, the future metapodium, to the left of the mid-ventral line (fig. 2, Plate 21). Its appearance, when first recognized in serial sections, is shown in fig. 31, Plate 26. It is secreted by the simple glandular ectoderm. BOUTAN, PATTEN and others do not appear to have observed the operculum in gastropod larvae until a much later stage than this, but ROBERT and SMITH saw the operculum rudiment in *Trochus* and *Patella* respectively, during torsion.

The mantle fold has extended so that it almost envelops the pedal rudiment. It has reached slightly more dorsally on the right side of the body than on the left, but otherwise the anterior end of the veliger is bilaterally symmetrical at this stage (fig. 31, Plate 26; fig. 46*A*). A very shallow groove, continuous with the mid-ventral mantle cavity, has developed by rapid multiplication of cells on both lateral extremities of

the mantle fold. As the pedal mass becomes more swollen, the pallial cavity separating it from the mantle fold gradually deepens (fig. 43). At the same time there is a marked dorsal constriction between the velum and the enlarging dorsal mass of digestive gland, where the umbo of the shell is commencing. The veliger has, therefore, a constricted "neck" region which is hidden ventrally by the pouch-like mantle fold (fig. 43).

Short apical cilia can be found in sections at this time. They are on two or three small cells in the centre of the apical plate (figs. 29 and 30, Plate 26). An hour or two afterwards they have disappeared. These were not discovered by MURAYAMA for *Haliotis gigantea*, and BOUTAN (1899) saw no apical cilia at any stage of *Haliotis* development. ROBERT found none in *Trochus*, but PATTEN (1886), WILSON (1904) and SMITH (1935) figure *Patella* veligers and trochophores with long cilia on the apical cells. If, as SMITH supposes, the telotroch serves as a rudder in *Patella*, its absence in *Haliotis* may be explained by the brevity of pelagic life.

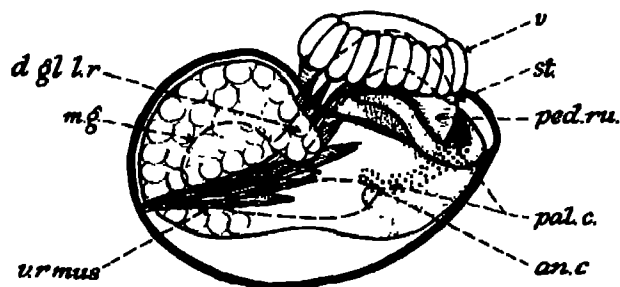


FIG. 43—Right side of veliger about 30 hr. old, immediately before torsion begins. From reconstructions made from serial sections, one of which is seen in fig. 25, Plate 25. The asymmetrical velum retractor muscle of the right side is shown. $\times 200$.

The visceral hump, which is posterior to the constricted "neck" region described above, is superficially bilaterally symmetrical, and in the works on other gastropod veligers, with the one exception of *Patella vulgata* (SMITH 1935), it has been assumed that this symmetry extends to the organogeny, but the serial sections of many veligers of *Haliotis* at this stage show that it is asymmetrical, as in *Patella*. The primitive digestive gland forms the whole of the left and most of the dorsal part of the visceral dome, and it has a conical anterior diverticulum on the dorsal left side. Immediately before pelagic torsion a second small conical process of the digestive gland grows towards the dorsal right side. It lies dorsally to the velum retractor muscle which passes to the right side of the body in this region (fig. 25, Plate 25; fig. 15, Plate 23). The asymmetrical position of the velum retractor muscle on the right side has already been described (p. 230); it is also shown in these two figures.

Pre-torsionally, the primitive mesoderm bands unite beneath the primitive rectum and, during the early part of torsion, the solid rudiments, which later form the coelomic walls of the kidneys and pericardium, develop in the region of the union of the mesoderm bands (fig. 42*b*). The renal rudiments remain solid until the late veliger stage is

reached, as in *Acanthochiton* (HAMMERSTEN and RUNNSTRÖM 1925) and in *Patella* (SMITH 1935).

In *Haliotis*, therefore, the two solid renal rudiments and the proctodaeum are the only representatives of the pallial complex when the early phase of torsion takes place. It may be gathered from SMITH's account that this is also true for *Patella*. It is important to note that in this respect *Haliotis* and *Patella*, which are undoubtedly primitive, depart from the theories of the mode of evolution of gastropod torsion, which are based on the assumption that the anus, the renal apertures, the ctenidia and the visceral loop were all established pre-torsionally. The full complement of these structures is not present in *Haliotis* until many weeks after torsion is complete.

FIRST 90° PHASE OF TORSION IN THE PELAGIC LARVA

This process was watched in the larvae resulting from fertilizations made on six different dates in July, August and September. It began while the veligers were swimming actively at a time varying from 29 to 35 hr. after fertilization. The torsion is a rotation in counter-clockwise direction, when the larva is regarded from its posterior aspect, and is a movement at right angles to the ano-pedal flexure.

Rapid rotation of 90° takes from 3 to 6 hr., after which the axis of the foot is situated at right angles to the median plane of the shell, so that only part of the margin of the shell is now ventral and the pallial cavity lies on the right side of the larva. The twist is made possible by, and localized to, the narrow neck-like region between the cephalo-pedal mass and the visceral organs enclosed in the shell (fig. 43 and fig. 16, Plate 23). Torsion begins as soon as the larval muscle cells have developed their retractile power. When these cells are contracted they become straight instead of curved (compare figs. 15 and 16, Plate 23). This would obviously force the newly formed "liver" diverticulum overlying the muscle towards the left side of the body. The contraction of the muscle pre-torsionally would also pull the enlarging pedal mass towards the exogastric shell. It is notable that the minute operculum rudiment has always appeared before torsion begins and is slightly to the left side of the foot (p. 231). Although it cannot be a very rigid cuticular plate at this time, it appears to collide with the left margin of the mantle and to act like a wedge pushing the mantle fold and shell margin away from the foot (fig. 2, Plate 21; fig. 31, Plate 26). It is therefore probable that the operculum is a contributory mechanical cause of the beginning of the rotation, although the contraction of the asymmetrical velum retractor muscle is the primary cause.

The attachment of the integument of the visceral mass to the shell on the pre-torsional left ventral side holds the visceral mass in position during torsion.

Resulting from this pelagic torsion the pallial cavity, containing the proctodaeum, is on the right side of the cephalo-pedal mass, which no longer hinders free entrance of water into the pallial cavity. The deepest part of the shell aperture is also to the

right and the umbo to the left, thus giving sufficient freedom for the foot and operculum until they are larger. The velum retractor muscle is now straight (fig. 16, Plate 23) and can retract the cephalo-pedal mass into the shell (fig. 3, Plate 21). The muscle is dorsal to the visceral mass, although its attachments are the same as before torsion begins. As a result of this first phase of torsion the weight on the two sides of the muscle is more symmetrically distributed. Prior to this 90° torsion the weight of the yolky digestive gland is nearly all on the left side of the body, giving an unstable equilibrium (compare fig. 25, Plate 25 with fig. 16, Plate 23 and fig. 27, Plate 25).

There is never more than 90° rotation of the pallial cavity at this stage in *Haliotis*, and it remains on the right side of the body for many days (figs. 46 and 48). The remaining 90° of torsion is a slow process of unequal growth spread over 8–10 days of ontogeny (figs. 46 and 48), and during most of this time the larva is benthic.

It is important to compare these observations of torsion in *Haliotis* with those of other authors. BOUTAN (1899) states that torsion begins when the shell is sufficiently developed to impede the spreading of the foot. This may be compared with SMITH's observation for *Patella vulgata* (1935) that growth of the foot produces an unstable condition. The possible influence of the development of the operculum is not mentioned by these authors. It is of particular interest to note that SMITH also holds the opinion that the action of the asymmetrical retractor muscle "plays a major part in bringing about torsion". His investigation of the torsion of *P. vulgata*, however, differs notably from the observations described above for *Haliotis* in that the pelagic torsion in *Patella* involves 180°, but in *Haliotis* 90° only. The diagram of the post-torsional larva of *Patella* shown in fig. 22*b* (SMITH 1935), however, scarcely appears to have undergone the complete 180° rotation of the pallial cavity, for the anal cell and visceral ganglia are to the right side. In *Patella* the first half of torsion takes about 40 hr., but the final stages are passed through quickly. In *Haliotis* the slow and rapid phases are reversed; the first half of torsion is accomplished in 3 or 4 hr., and the second half takes many days to complete. BOUTAN (1899) states that 180° torsion is completed in 2 or 3 min. in *Haliotis* and almost as rapidly as in *Acmaea*. He did not examine the succeeding stages in sections, and he was unable to determine the length of larval life. Without transverse sections and models of such minute larvae it is impossible to get an exact idea of the amount of torsion accomplished.

TRANSITION FROM PELAGIC TO BENTHIC LIFE

Bionomics of Young Plantigrade

This period begins when the veliger is 2 days old and ends when it is from 10 to 14 days old. As in lamellibranchs, the pelagic life is brief and, when 40 hr. old, the larvae rest at the bottom between intervals of swimming. These intervals shorten and life is entirely benthic after the 3rd day, but the dorsal part of the velum is not com-

pletely thrown off until nearly 2 weeks after fertilization. The changes in appearance of living larvae during this transitional period are shown in figs. 7-14, Plate 22.

Feeding does not begin until the larvae cease to be entirely pelagic. Veligers from 40 hr. old can make use of a mixed culture containing *Pleurococcus* and other organisms smaller than *Nitzschia*. At this time there are long mouth cilia which aid the long velum cilia in wafting food into the mouth. Feeding continues in this manner during creeping so long as the velum persists. From 8 to 14 days old the larvae can make use of *N. closterium*, which is frequently found in transverse sections passing through the larval stomach.

The teeth of the radular ribbon and the two first formed supporting cartilages are only sufficiently developed for use by the time the velum disappears at the end of 2 weeks of development (fig. 33, Plate 27). At first the radula scrapes up loose fragments, such as diatoms and foraminifera, from the surface of the stones on which the larvae creep. The shell is tinged with pink and the soft parts of the body are faintly green, so that the young creeping animals resemble their food in colour.

The pedal sole is first seen at the end of the 3rd day of development as an invagination on the antero-left surface of the foot. The area of the pedal sole rapidly increases and a carpet of short and very active cilia clothes the epithelium (figs. 7 and 9, Plate 22). When the veliger retracts the foot is folded so that the pedal sole is completely hidden.

The first attempts at creeping were watched at the close of the 3rd day of development. The early efforts of these veligers met with little success. Larvae which were lying on their sides in a resting contracted state inside the shell suddenly began to open the shell aperture by rotating the operculum towards the shell umbo (figs. 7, 8, 9 and 10, Plate 22). During this rotation the mobile foot spread its pedal sole gradually, first concave and then flat and the larva attempted to cling by the plantar surface. The posterior part of the pedal sole seemed to touch and obtain a hold first (figs. 8 and 12, Plate 22). The anterior part then moved in a stretching and searching manner (fig. 11, Plate 22). In larvae 3 days old, however, the foot seemed unable to get a suitable hold for creeping. This was apparently because of difficulty in getting into equilibrium the weighty visceral hump over the foot. Obviously the relation of the visceral hump to the force of gravity had changed when creeping was attempted. The larvae fell sideways and retracted into resting position, usually lying on the left side because the shell margin is shorter on this side (figs. 8 and 9, Plate 22).

Further attempts at creeping were made after a considerable period of rest, but they were not successful until the larvae were between 4 and 5 days old, when the pedal sole was considerably larger and, as sections show, had well developed muscle strands. An attempt has been made, in figs. 7-14, Plate 22, to show the appearance of the larvae during these creeping efforts but, as BOUTAN noticed, the larvae are difficult to observe because they are very sensitive and rest for prolonged periods. A larva $4\frac{1}{2}$ days old struggled for 10 min. after lifting its operculum and clinging with its foot. There was much elongating, stretching and swinging in the "neck-like"

region between the cephalo-pedal mass and the visceral hump enclosed in the shell and in the now constricted region above the foot (fig. 9, Plate 22). As the shell with its contents was swung from side to side, apparently in an attempt to get it into equilibrium, the operculum appeared to collide with the shell (figs. 9 and 10, Plate 22). Finally, with the operculum and metapodium directed to the right side and the visceral hump leaning a little to the left, as shown in fig. 13, Plate 22, creeping was successful for about 40 min. The larvae progressed in a gliding manner, by the aid of the very active cilia of the pedal sole, the latter presumably being lubricated by the mucus of the pedal epithelium. During the 6th and 7th days, the larvae were visible to the naked eye creeping on the surface of flat stones. They were also seen to make use of the surface film. The habit of creeping beneath stones was not acquired until after the first fortnight of development.

Larvae of *Patella vulgata* (SMITH 1935) began to crawl on the 9th day, but they were not entirely sedentary until about 15 days old.

Pedal Glands

From the 3rd day of development there are large unicellular mucous gland cells scattered in the pedal epithelium (fig. 34, Plate 27). In the metapodial region, where the operculum is secreted, these gland cells are more spherical than those of the pedal sole. The operculum appears to be secreted by these simple gland cells, for no special foot organ, such as is found in *Patella* (SMITH 1935) in relation to secretion of the operculum, is present in *Haliotis*. In the 7-day-old plantigrade there is, however, a large anterior pedal gland. It is an aggregation of very large cells of mesodermal origin, which stain deeply with aniline-blue as do the epidermal mucous cells. This gland lies close to the pleuro-pedal ganglia and appears to discharge into a small depression of the anterior end of the foot beneath the mouth (fig. 33, Plate 27). It is a simpler gland than the pedal glands of *Patella*.

In the oldest veligers there are also pedal mesoderm cells of very large size and vacuolated appearance, which resemble the cells of unknown fate in *Patella* (SMITH 1935); they cannot be distinguished from the lymphoid cells of the adult *Haliotis* (CROFTS 1929, p. 100).

Changes in the Velum

During the 3rd day a vertical groove forms in the prevelar area. This extends ventrally to make a cleft in the velum in the region of the mouth (fig. 6, Plate 21). BOUTAN (1899) states that in *Haliotis* he is unable to determine whether the velum remains entire or indents, as in the later stages of *Fissurella*.

The age at which the velum begins to diminish varies from 7 to 10 days in different individuals. The velum cells decrease in size and are gradually nipped off, as in *Patella* (SMITH 1935), by the tegumentary cells of the head growing beneath them (fig. 35, *integ.v.c.*, Plate 27). In *Haliotis* the velum disappears from the right side before the

left (fig. 33, *v.v.*, Plate 27). This may be due to development of the large mantle fold on this side. In some larvae at 14 days there are still velar cells although the velum has entirely disappeared in other larvae at 10 days old.

Sense Organs

The sense organs of *Haliotis* begin to develop at about the same stage as in *Trochus* (ROBERT 1902). In *Paludina*, according to ERLANGER (1891) and DRUMMOND (1902), the rudiments of the sense organs arise when development in other respects is much less advanced than in *Haliotis*. This may be due to abbreviation of the developmental stages in *Paludina*, as a result of viviparity.

The single pair of cephalic tentacles develops from small prominences on the lateral region of the enlarged part of the pre-velar area. These rudiments are first recognizable in veligers about $2\frac{1}{2}$ days old (fig. 6, Plate 21). At first they point dorsally, but when the larvae are 4 days old they point ventrally, having elongated and become mobile (fig. 9, Plate 22).

During the 4th day there is pale green pigment in the epithelium of the tentacles. At this time they develop papillae, the larger of which bear terminal tufts of cells with rigid sensory threads (figs. 7-14, Plate 22), the taste-buds or "Pinselzellen" of FLEMMING (1883). The papillae do not increase in size but their number is gradually increased until, in the post-larval stages, they are finally arranged close together and give the appearance of velvet pile covering the whole surface of the tentacles. Innervation of the cephalic tentacle by an outgrowth of the cerebral ganglion is shown in fig. 35, *ceph.t.n.*, Plate 27.

The rudiments of the eyes are first seen early in the 3rd day as a group of several epithelial cells, which have acquired pigment granules, situated near the velum on the outer sides of the cephalic tentacle rudiments (figs. 5 and 6, Plate 21). Before the close of the 3rd day these eye rudiments are raised on small projections of the base of the tentacles, which are the rudiments of the optic tubercles (fig. 17, Plate 23). Owing to thickening of the epithelium, the pigmented cells begin to sink in and form a shallow retinal invagination. In the sections of larvae a week old, the retinal epithelium is in the form of a cup and is innervated by a process from the cerebral ganglion, which, like that innervating the tentacle, is ganglionated (fig. 35, Plate 27).

The retinal cells have begun to secrete cuticular outgrowths to initiate the retinidian layer and the crystalline lens (fig. 35, Plate 27). This refractive body grows large enough by the end of the 2nd week to block the opening of the cup and to project at the tip of the optic tubercle. The ocular cup never becomes closed, so that the eye does not acquire a cornea and in this respect the eye is more primitive than that of many members of the Archaeogastropoda. It was not found possible to trace the development of different types of retinal cells, which are described for the adult (CROFTS 1929, fig. 24, Plate VI).

The statocyst rudiments are first seen in veligers about 30 hr. old, which have

undergone 90° torsion. They arise as a pair of shallow invaginations of the ectoderm near the junction of the latero-dorsal part of the foot with the head (fig. 30, Plate 26 and fig. 5, Plate 21). They are farther from the mouth than PATTEN (1886) describes them for *Patella*, but SMITH (1935) shows that PATTEN had mistaken the precocious mantle cavities for statocysts. In *Haliotis* during the latter half of the 2nd day these invaginations sink into the foot and by the 3rd day the statocysts are cut off from the pedal ectoderm (fig. 44*b*). The spherical statocysts are now embedded in the foot and are situated close to the rudiments of the pleuro-pedal ganglia (fig. 32, Plate 26). They remain adherent to these nerve centres although they have no physiological connexion with them. They become innervated during the 3rd day by nerve cells connected with the cerebral ganglia, which are but a short distance from the statocyst in these minute veligers (fig. 44*b*). The branched sensory processes of the statocyst

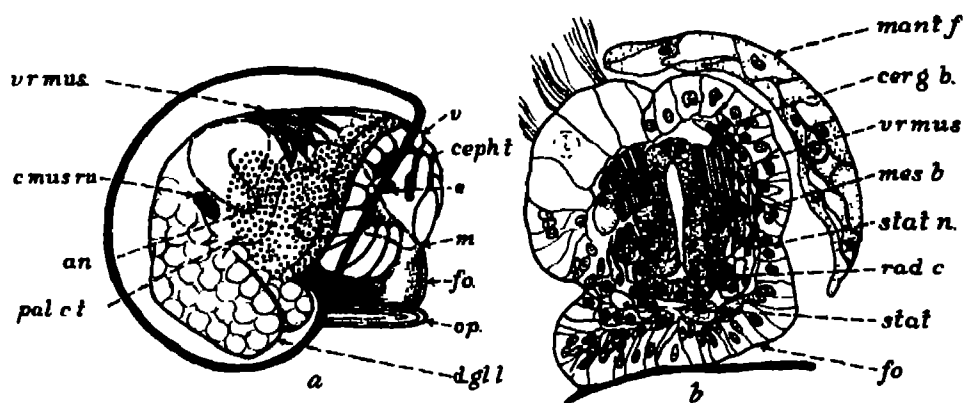


FIG. 44—*a*, veliger 61 hr. old, seen from right side, drawn from reconstruction; pallial cavity and anus show 90° torsion. Rudiment of columellar muscle also on right; velum retractor muscle partially migrated to left. *b*, transverse section at same age, passing through rudiment of radula and showing innervation of statocyst from cerebral ganglion. $\times 450$.

epithelium, which are seen only with an oil immersion lens, are present in older veligers. One statolith only is secreted in each statocyst, but in the post-veliger there are additional statoliths.

According to DRUMMOND (1902) *Paludina* has its statocysts already enclosed in the foot some time before torsion begins. In *Patella* they are formed later and, although not quite so early as PATTEN (1886) imagined, just before torsion begins (SMITH 1935). It has been shown above that the statocyst invaginations of *Haliotis* do not develop until the pelagic half of torsion is accomplished.

Muscles

The development of the muscles, which has been followed in serial sections and reconstructions, is of particular interest since it differs from the generally accepted hypothesis of the mode of origin of gastropod musculature.

The velum retractor muscle develops pre-torsionally and its significance in relation

to the first half of torsion has been described (pp. 233 and 234; figs. 15 and 16, Plate 23; fig. 27, Plate 25). At the end of this pelagic phase of rotation this muscle is dorsally situated and during the 2nd day of development it can withdraw the cephalo-pedal mass completely into the shell. In the contracted state shown in fig. 17, Plate 23, the velum retractor muscle is a stout short pillar inserted under the velum and passing postero-dorsally to its shell attachment. The position of the attachment is now displaced slightly to the left because of the enlargement of the primitive digestive system and of the

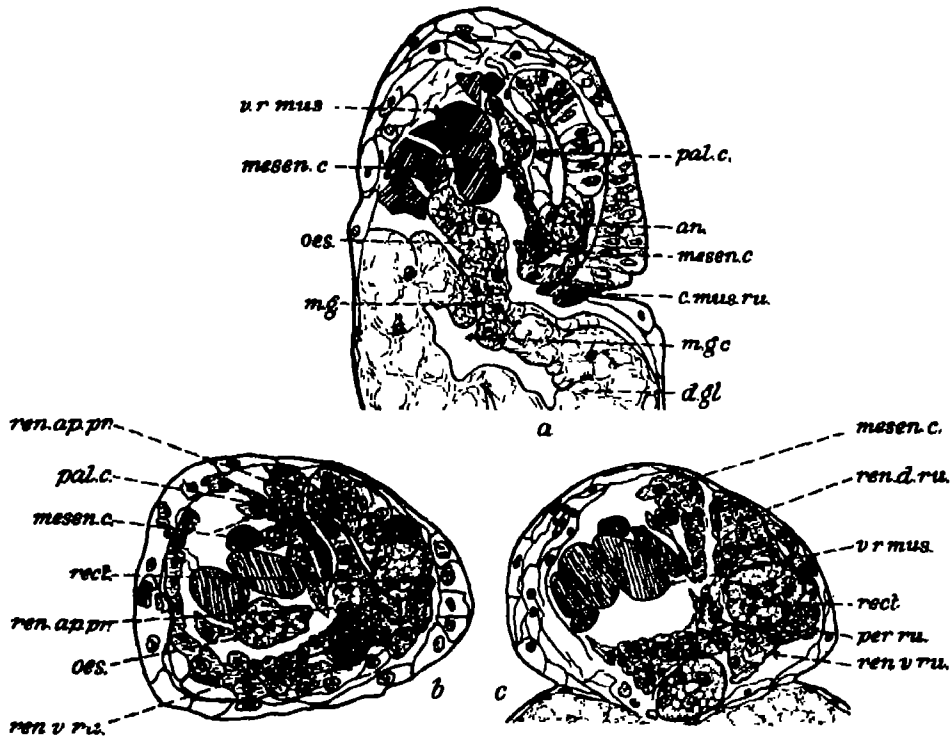


FIG. 45—Transverse sections of veligers 61 hr. old, passing through posterior end of pallial cavity. $\times 450$. *a*, shows rudiment of columellar muscle on right, ventral to pallial cavity and mesoderm band. *b*, from another series, showing diverticula of posterior end of pallial cavity (precocious renal apertures); solid mesoderm rudiments of renal walls dorsal and ventral to anus. *c*, from same series as *b*, shows renal rudiments united by solid mesenchyme rudiments of pericardial wall.

pallial region on the posterior right side. In 3-day-old larvae the velum retractor muscle occupies most of the dorsal left side of the visceral hump and is bordered on the right side by the pallial cavity anteriorly and the primitive gut posteriorly (fig. 45*a* and fig. 17, Plate 23).

Further change in position of the muscle attachment to the shell takes place in veligers from 4 days old and is brought about both by migration and by addition of new muscle cells from the mesoderm of the left side. As the shell grows, the muscle attachment moves in an antero-left direction so that it becomes progressively nearer the left shell margin (figs. 7, 9 and 12, Plate 22; figs. 17 and 18, Plate 23). In these

creeping veligers the velum retractor muscle develops spindle-like muscle cells extending into the left side of the foot (fig. 35, Plate 27). Thus it serves as an initial, if somewhat inadequate, pedal retractor.

When the velum is in process of disintegration, at an age varying from 9 to 14 days, the velum retractor muscle ceases to grow. It is now inserted on the left half of the developing buccal apparatus and its shell attachment is close to the small mantle fold, which has now extended round the left side. The topographical relations are shown in transverse sections and in the figure drawn from a reconstruction of a larva 14 days old (figs. 33 and 34, Plate 27; fig. 18, Plate 23). Muscle cells in the dorsal part of the foot connect the velum retractor muscle with the enlarging columellar muscle (fig. 34, Plate 27).

The columellar muscle develops from two mesoderm cells of the pre-torsional left side (fig. 25, Plate 25 and p. 230), which are retarded in development until the creeping habit begins. These two cells are recognizable in sections of a number of larvae after the first phase of torsion and they are shown in the transverse sections of a veliger at 61 hr., lying under the groove between the pallial cavity and the digestive gland on the right side of the visceral dome (figs. 44*a* and 45*a*). At this time these mesoderm cells are insignificant in comparison with the velum retractor muscle cells, but they elongate and multiply and in larvae 6 days old have formed muscle cells running into the metapodium and its opercular process, which has developed on the right side. The columellar muscle and its shell attachment, near the right side of the shell umbo, are shown in a 6-day larva in fig. 8, Plate 22.

When the velum begins to disintegrate, at an age varying from 9 to 14 days, the columellar muscle is still much smaller than the velum retractor muscle. The columellar muscle lies to the posterior right side of the foot, and has only a small attachment to the shell (fig. 18, Plate 23). In addition to the opercular muscle strands, which are derived from mesenchyme and are inserted under the opercular epithelium, muscle processes are also added to the columellar muscle in the centre part of the foot. Some of these processes are attached under the epithelium of the pedal sole (fig. 34, Plate 27).

In larvae, which have lost almost all the velum, the two muscles are approximately equal in size, for the velum retractor muscle has now ceased to grow. The latter muscle is situated on the antero-left side and the columellar muscle is on the postero-right side (fig. 19, Plate 24; fig. 46*E*). They function in directions approximately at right angles to one another, the velum muscle contracts in an antero-posterior direction, thus retracting the head, and the columellar muscle contracts in a dorso-ventral direction withdrawing the foot into the shell. With the change in topographical relations when benthic life begins, it seems probable that the columellar muscle is more effective in helping to preserve the balance of the visceral hump over the foot during creeping than is the velum retractor muscle on the left side. From this time the columellar muscle becomes the important muscle, the velum retractor muscle becoming less and less significant.

It has already been shown (p. 231) that the velum retractor muscle of *Haliotis* closely resembles the dorsal retractor muscle of *Patella vulgata* (SMITH 1935). From the evidence of *Haliotis* pre-torsional and post-torsional stages, however, it appears probable that originally the veliger retractors were paired and that the columellar muscle develops

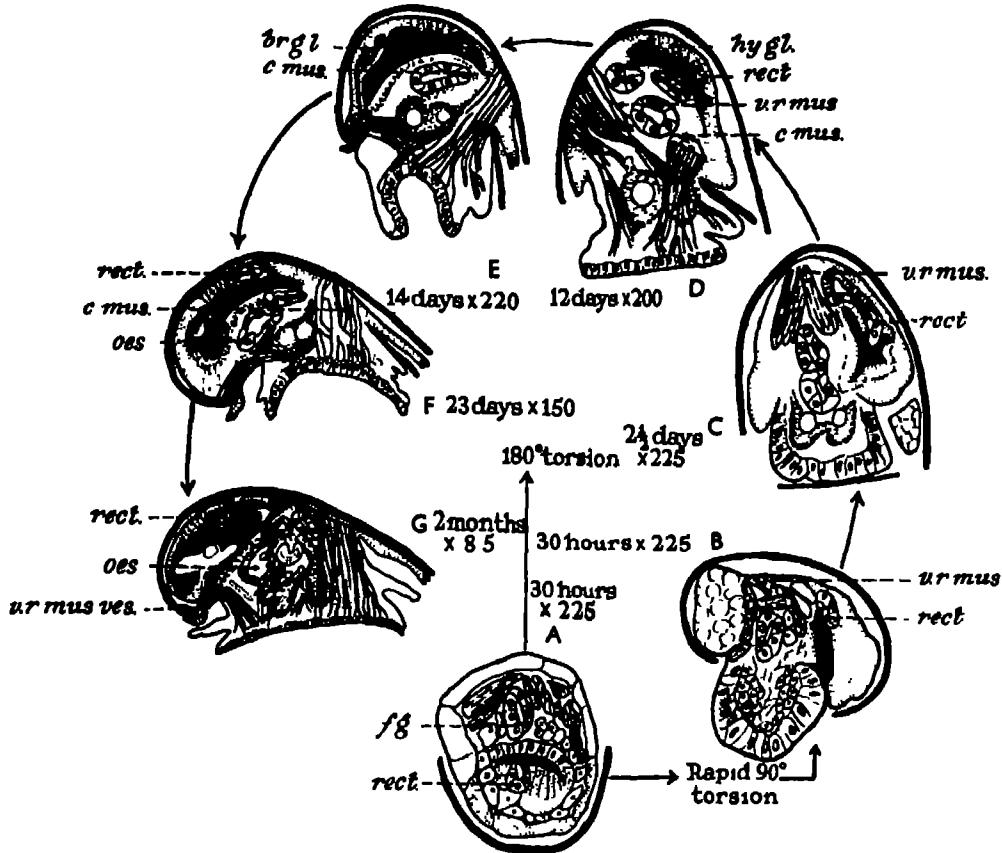


FIG. 46—Diagrammatic transverse sections of veligers and post-veligers to show development of muscles, pallial complex, and migration of the pallial cavity. Right side of animal towards right side of page, pallial cavity shown in black, mantle fold stippled, shell in heavy outline, muscles striated. A, immediately prior to torsion, through posterior part of pallial cavity. B, after rapid 90° torsion. C, at 2½ days with pallial cavity still on right side. D, oldest with velum, pallial cavity almost dorsal with hypobranchial gland and branchial ganglion rudiments, two muscles equal in size. E, early post-veliger with rectum still on right side of pallial cavity; supra-oesophageal pleuro-visceral cord dotted. F, post-veliger (23 days old); first ctenidium, final left, now developing lamellae; velum retractor muscle inconspicuous. G, at close of metamorphosis (2 months old) well-developed final left ctenidium and rudimentary final right ctenidium.

from one member of the pair, which is arrested in development until creeping is attempted. Although SMITH states that the larval retractor muscle cells disappear after pelagic life ceases and that the adult shell retractors have an entirely different origin, the rudiment of the adult shell muscle of *Patella vulgata* (fig. 22 a, SMITH 1935) closely resembles the rudiment of the columellar muscle of *Haliotis* in position and in

its function (figs. 8 and 11, Plate 22). For *Haliotis* it is impossible to agree with SMITH's theory, which is also the generally stated one, that the functional retractor muscle of the pelagic larva is homologous with the columellar muscle of spirally twisted gastropods. Although the veliger retractor muscle of *Haliotis* is retained, unlike that of *Patella* (SMITH 1935), it becomes an insignificant shell muscle and is not the columellar muscle. Its fate in the post-veliger is described on p. 252 and its final position is shown in fig. 22, Plate 24 and fig. 38, Plate 27.

In *Haliotis* there is no corresponding muscle to the ventral retractor which appears after torsion in *Patella*, although pre-torsionally there is a simple tegumentary attachment of the visceral hump to the shell in postero-left position (fig. 3, Plate 21).

Mantle and Shell

The mantle and its derivatives change much during the transition to benthic life. As a result of the first torsion phase, the shell is half endogastric (figs. 3 and 4, Plate 21; fig. 16, Plate 23). The deepest part of the shell, which coincides with the deepest part of the mantle fold, is on the right side of the body with a slight tilt dorsally because, pre-torsionally, it was a little to the right side (p. 231). The umbo is therefore on the left ventral side of the body (fig. 16a, Plate 23). There is now room for growth of the mantle and shell on the right and right-ventral side. The foot and operculum have freedom for movement which is adequate for a few hours.

During the late part of the 2nd and the early part of the 3rd day, semblance of an endogastric shell is brought about gradually by retardation of shell growth due to the movement of the enlarging foot and operculum over the ventral shell margin as the larva moves in and out of the shell. The umbo, therefore, spreads from the left towards the right side (figs. 3 and 4, Plate 21). The asymmetrical increase to the shell margin is shown in fig. 47. Examination of the shell alone gives the misleading appearance of 180° torsion with a completely endogastric shell (figs. 5 and 6, Plate 21). Figs. 44 and 45a show that the mantle cavity, containing the anus, is still on the right side of the larva. A very shallow mantle fold extends dorsally to the left side (fig. 17, Plate 23).

Rapid growth of the margin of the shell gives a more rolled character, so that, at about 60 hr., the shell can accommodate the retracted veliger completely (fig. 17, Plate 23). The operculum is now large enough to close the shell aperture.

During the 3rd day the shell shows curved lines radiating from the umbo to the margin and on these are glistening specks, which are probably carbonate of lime deposited to strengthen the original shell of conchiolin.

The foundation of the dextral coil of the shell can be detected from the 3rd day, in a more rapid addition to the shell margin on the right side than on the left (figs. 8 and 9, Plate 22). This appears to be due to extensive proliferation of the cells of the deep mantle fold overlying the pallial cavity, which remains entirely on the right side of the body until after the 6th day of development, when creeping has become

established (figs. 44, 45 and 46). This results, during these 4 days, in additions to the shell mainly on the right side so that the shell leans towards the left side during creeping. An additional cause of this tilt of the shell and its contents may be that the left half, containing the velum retractor muscle and the digestive system, is heavier than the right half, which is largely occupied by the pallial cavity (fig. 18, Plate 23).

During late veliger development the right mantle fold gradually surrounds the posterior part of the columellar muscle and joins the smaller left mantle fold beneath the visceral hump, so that the original umbo is lifted above the new peristome (figs. 11 and 14, Plate 22).

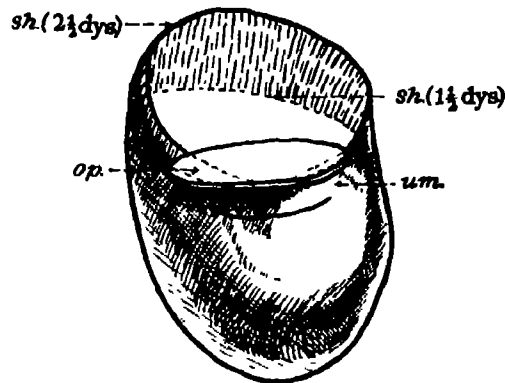


FIG. 47.—Diagram of ventral view of reconstruction of shell to show its asymmetrical growth after 90° torsion. $\times 200$ approx. Shell margin immediately after 90° torsion, when shell is half endogastric, is indicated. Outer shell margin is at $2\frac{1}{2}$ days old, when shell is completely endogastric, although pallial cavity has not yet undergone 180° torsion. *op.*, position of operculum; *um.*, umbo.

Gradual Completion of Torsion of the Pallial Cavity

At the time the creeping habit is established in larvae about 6 days old, the second 90° of torsion of the pallial cavity has begun. This is accomplished mainly by a gradual migration, due to differential growth, but conceivably the leaning of the shell and its contents to the left side, which is mentioned in the preceding section, contributes to the rotation. The process is not completed until about a week later, when the early post-veliger stage is reached.

It has been shown in the account of the muscles (p. 239) that the larval retractor muscle gradually migrates to the left side during late veliger development. At the same time, the columellar muscle migrates from the right side towards the centre of the shell and, in contrast to the velum retractor muscle, it gains in size and functional significance (compare fig. 18, Plate 23 with fig. 19, Plate 24 and fig. 46, D and E). Thus it is clear that more and more room is left for the pallial cavity to expand dorsally from the right side of the body, but that the development of the columellar muscle prevents spreading from the right border of the cavity. Therefore, in the late veligers and early post-veligers, the gradual expansion of the pallial cavity concerns only that part which is to the left side of the rectum (fig. 36, Plate 27).

By the time the velum is lost, at not earlier than 12 days old, the pallial cavity is dorsal and has undergone 180° torsion from its original position, but the anus is still on the right side of the pallial cavity (fig. 19, Plate 24; fig. 48E).

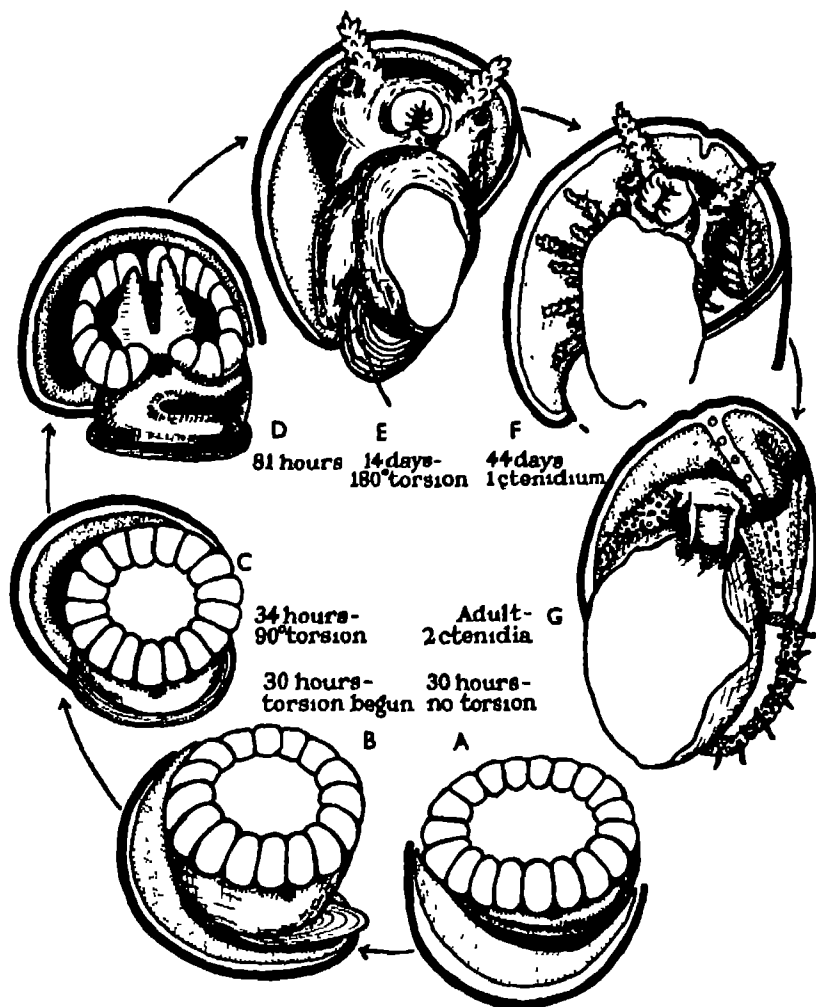


FIG. 48—Anterior views to show phases of torsion and later displacement of the pallial cavity. Pallial cavity shown in black, mantle fold stippled, velum unshaded, foot cross-hatched, operculum with concentric curves. A, B and C show sudden migration of pallial cavity through 90° , from ventral position to right side. 180° torsion is completed slowly after about 14 days. E shows dorsal pallial cavity. F and G show displacement of pallial cavity to the left side by hypertrophy of columellar muscle. G shows adult with pallial cleft, bilateral symmetry of pallial complex and shell holes placed dorsally to anus.

The Pallial Complex

The pallial complex, which in the adult is constituted by two ctenidia with their osphradia and hypobranchial glands, the median anus and renal openings on either side of it, is not complete until the end of nearly 2 months of development (figs. 21

and 22, Plate 24). The relations existing between these structures and the mantle and shell are of the highest importance in Mollusca, therefore the order of development of these organs has been investigated. It has already been shown on p. 233 that, at the time of the first half of torsion, the proctodaeum and the solid mesodermal rudiments of the kidneys are the only representatives of the complex. The following account will show that, by the time 180° rotation of the pallial cavity is complete, the rudiment of one ctenidium only is present. The corresponding osphradium and hypobranchial gland have appeared in addition to the anus and two kidneys which have acquired cavities and apertures.

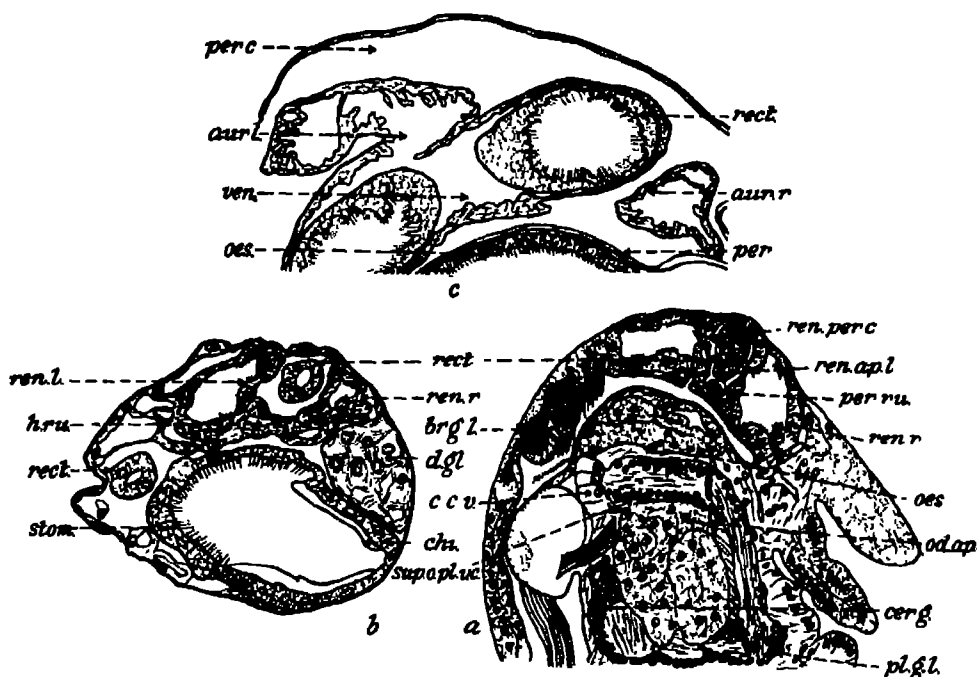


FIG. 49—*a*, parasagittal section of late veliger viewed from right; definitive left kidney now mid-dorsal and definitive left one on right side; mesenchyme cells migrating to form pericardial wall, which envelopes rectum. Epithelial cells about to cut off velum cell. $\times 450$. *b*, transverse section of youngest post-veliger, immediately posterior to pallial cavity, two adjacent sections combined; shows two kidneys in post-torsional position and rectum surrounded by pericardial coelom and heart rudiments. $\times 450$. *c*, transverse section of metamorphosed *Haliotis* at 2 months showing heart, with two auricles, enveloping rectum and surrounded by pericardium. $\times 100$.

Renal organ rudiments. The kidney rudiments, as seen in sections of various 3rd-day veligers, are still solid masses of mesenchyme cells, such as were described for the pre-torsional veliger (p. 232), but situated dorsally and ventrally to the rectum and attached by the solid pericardial wall rudiment close to the rectum (fig. 45*c*).

At the time reduction of the velum has begun, the rudiments of the renal cavities develop as spaces in the renal rudiments, which are now on either side of the rectum. Fig. 49*a* shows the renal organs in a transverse section of a larva whose velum has almost gone. The two kidneys are almost equal in size and are lined by ciliated cubical

cells. In the earliest post-veliger they are in post-torsional position (fig. 49*b*). The mesenchyme cells nearest the rectum are now arranged to make a delicate coelomic wall of small irregularly disposed cells surrounding the rectum and enclosing the pericardial cavity next to the rectum (fig. 49*a* and *b*). In having the pericardium traversed by the rectum *Haliotis* resembles the lamellibranchs and differs from *Patella*. When first the pericardial rudiment forms, the reno-pericardial canals are obvious and the left one is shown in fig. 49*a*. They were not found in *Patella* (SMITH 1935).

The renal apertures appear to arise as precocious rudiments, which are seen late in the 3rd day after fertilization as simple ectoderm invaginations in the deepest part of the pallial cavity. They can be recognized only in sections; the post-torsional left one lies dorsally to the anus and the post-torsional right one is ventral to it (fig. 45*b*). These invaginations do not communicate with the renal organs until about 9 days later, when the velum has disappeared. Although the cells lining the renal cavities possess short cilia (fig. 49*a*), the renal apertures are not ciliated like those of *Patella* (SMITH 1935).

In the early post-veliger, torsion is complete and the renal organs are now to the right and left of the anus, as in the adult (fig. 19, Plate 24; fig. 49*b*).

The classic account of ERLANGER (1891, 1894) and that of DRUMMOND (1902), which are mainly concerned with the coelom and its derivatives, show that in *Paludina* the kidneys and their pallial apertures take part in 180° of torsion. Pre-torsionally, however, organogenesis is more advanced in *Paludina* than in *Haliotis*, since in the latter the rudiments of the renal cavities and of their apertures are obvious only after the first half of torsion, although the mesoderm from which the kidneys are formed undergoes 180° torsion. It may be noted that DRUMMOND repeats HALLER's erroneous statement that there is only one renal organ in *Haliotis*.

First ctenidial rudiment. The ctenidial and osphradial rudiment of the final left side alone is developed in the veliger. At the 7th day it is a thickened epithelial ridge in the roof of the pallial cavity to the left side of the anus but posterior dorsal to the right eye. In sections the branchial ridge is seen thickly clothed with long cilia, which, even in the oldest veliger, is a simple small ciliated band in the roof of the pallial cavity (fig. 35, Plate 27 and fig. 18, Plate 23). This ctenidial rudiment remains a simple ciliated band for about a week.

A small part of the mantle prominence, which bears the ctenidial rudiment, has shorter cilia and is the osphradial rudiment of the definitive left side. The position of this rudiment relative to the branchial ganglion is shown in the transverse section of an early post-veliger in fig. 36, Plate 27.

First hypobranchial gland. The final left hypobranchial gland develops in the roof of the respiratory chamber of the late veliger. It is very small in sections of 12-day-old larvae with reduced velum, and is situated dorsally between the rectum and the first ctenidial rudiment (fig. 46*D*; fig. 36, Plate 27). Already this gland has large mucous cells and small ciliated cells.

Digestive System

The description of the pre-torsional veliger includes an account of the development of the primitive gut and its flexure (figs. 41, 42 and 43; figs. 15 and 15*a*, Plate 23).

After the first half of torsion, in veligers 2 days old, the stomodaeal invagination is elongated into the foregut, which now communicates with the short oesophagus. The latter, like the primitive stomach, the digestive gland rudiment and the primitive intestine, has indistinct limits. The digestive gland lies mainly on the left side of the visceral dome, with a dorsal conical process pointing anteriorly (figs. 26 and 27, Plate 25; figs. 28 and 29, Plate 26). At this stage it has only one stomach orifice, which is to the left side of the primitive stomach (fig. 16, Plate 23). In the late veligers, which can retract completely into the shell, the digestive gland is able to change its shape considerably to fit in with the other organs when they are contracted. At 60 hr. the two anteriorly directed digestive gland processes are in ventral position, so that there is room in the dorsal part of the shell for accommodation of the velum and developing sense organs (figs. 44 and 45*a*). In contracted specimens at this stage there is a groove between the right ventral part of the digestive gland and right dorsal pallial cavity.

In veligers a week old the intestine has elongated, the intestinal cells are now smaller and have acquired cilia. The anus opens into the most posterior part of the pallial cavity (fig. 17, Plate 23). During the 2nd week the intestine is further elongated and is thrown into two short loops so that it can be accommodated in the dorsal part of the visceral hump. The anus still opens on the right side of the body as it did immediately after the first half of torsion, but the pallial cavity is now extended considerably on the dorsal side of the anus (fig. 18, Plate 23 and fig. 33, Plate 27).

In the oldest veligers the larval stomach has enlarged so that it reaches the extreme posterior end of the body (fig. 18, Plate 23).

The rudiment of the radular caecum is first seen after the first phase of torsion, in veligers about 40 hr. old. In this respect *Haliotis* differs from *Paludina* (DRUMMOND 1902) and *Patella* (SMITH 1935), in which it is already obvious pre-torsionally. This caecum arises as a simple ventral diverticulum of the foregut (fig. 44*b*). During the succeeding days it enlarges until, in 5-day veligers, it has become the radular sheath in which the radular ribbon is secreted by the epithelium at the extremity of this sheath. At 7 days the epithelium has secreted several rows of teeth and, as in *Patella* before the end of metamorphosis (SMITH 1935), there is a central tooth in each row. In the early stages of metamorphosis in *Haliotis*, however, there are three lateral teeth on either side of it (fig. 33, Plate 27) instead of one as in *Patella*. Before the velum has disappeared the radular ribbon begins to function. It appears to catch up loose diatoms, etc., since these are found in sections through the stomach. There is no evidence of fragmentation of seaweed at this stage.

Nervous System

The development of the nervous system was followed from transverse sections and from six reconstructions of the nervous system and related digestive system, which were removed from the wooden reconstructions of whole larvae of different ages.

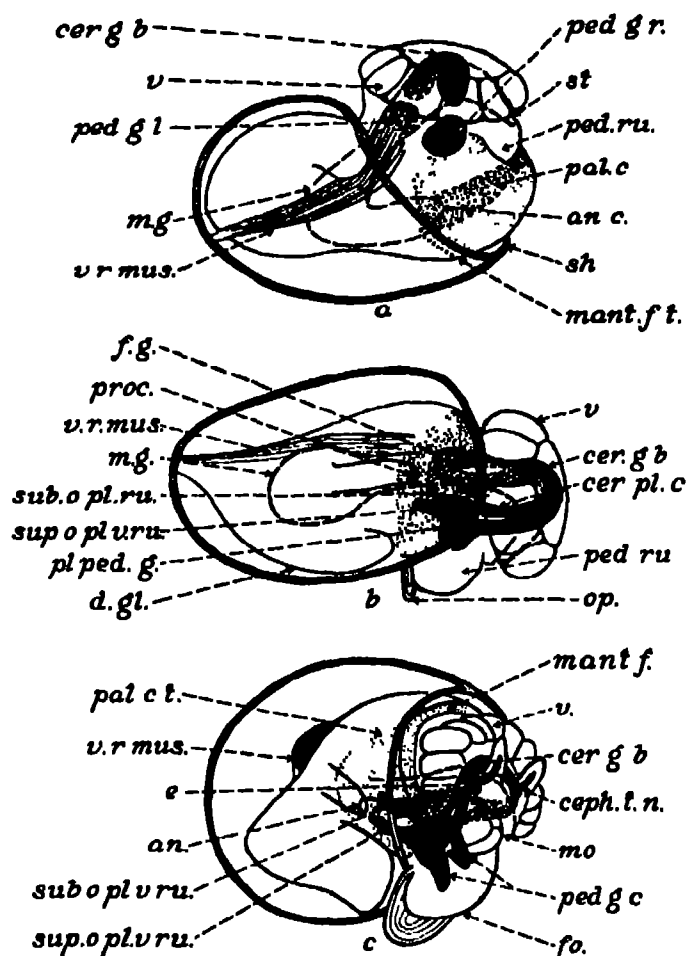


FIG. 50—Diagrams to show the development of the nervous system. Right side views from whole mounts and reconstructions. Nervous system indicated in black for right side and cross-hatched with circles for left side. Size of nervous system somewhat exaggerated in *a* and *b*. Pallial cavity stippled. *a*, Pre-torsional veliger; cerebropedal nerve ring almost delaminated. *b*, Veliger immediately after 90° torsion. Rudiments of cerebral, pedal and pleural ganglia are shown and pleuro-visceral processes are very rudimentary. *c*, Veliger 3 days old. Optic and tentacular nerves, pedal ganglia have begun elongation to pedal cords; fusion of pleural and pedal ganglia; right pleuro-visceral process short, left one already sub-oesophageal.

It has already been shown that, in the pre-torsional stage, the cerebral ganglia alone are delaminated and the pedal ganglia are in process of delamination (p. 231). The rudiments of the pleural ganglia are first noticed before the pelagic half of torsion is completed. They form a collection of sinking ectoderm nuclei situated dorsally to

the pedal ganglion rudiments, but on either side of the head near the most lateral parts of the mantle fold (fig. 31, Plate 26). Before the end of the 2nd day after fertiliza-

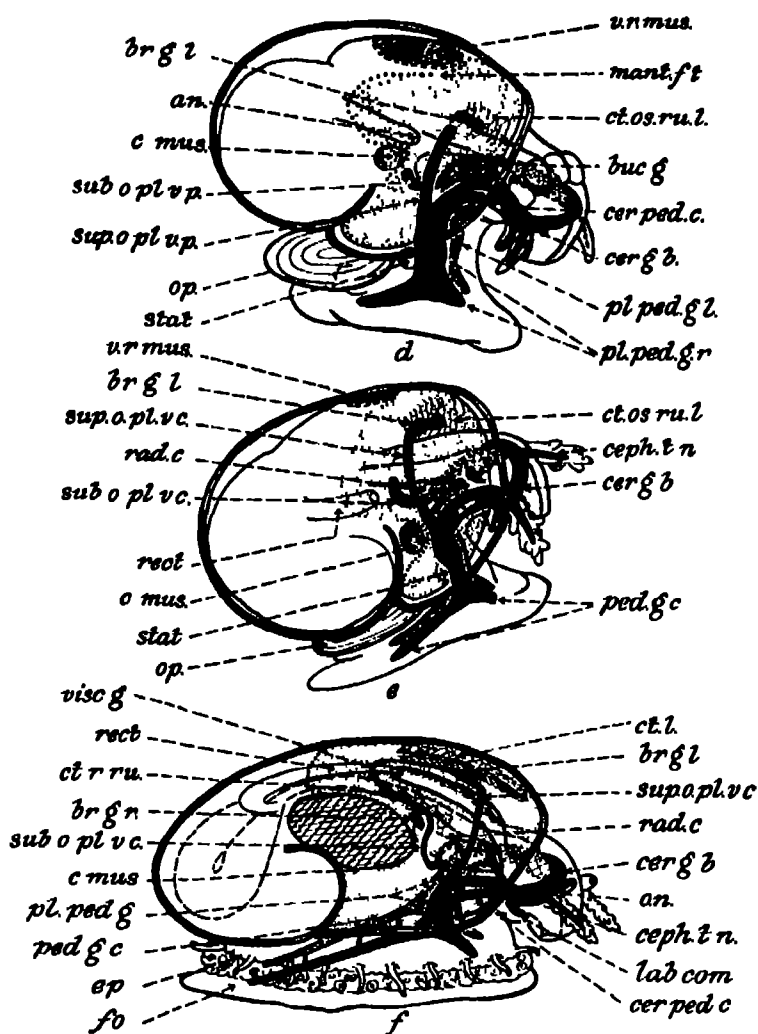


FIG. 51.—Continuation of diagrams to show development of the nervous system. (For simplification the labial commissure is omitted.) *d*, Oldest stage with vestiges of velum (12 days); one branchial ganglion, definitive left, in dorsal part of pallial roof but not yet united to supra-oesophageal pleuro-visceral cord. Rudiment of definitive left osphradium and ctenidium indicated near branchial ganglion. *e*, Post-veliger soon after loss of velum (14 days); supra-oesophageal pleuro-visceral cord now united with its branchial ganglion. *f*, Post-veliger at 2 months old; metamorphosis complete; definitive right ctenidium developing and giving bilateral symmetry of pallial complex; sub-oesophageal pleuro-visceral cord has acquired a branchial ganglion; visceral part of the pleuro-visceral loop has developed.

tion the pleural ganglia are delaminated. They lie close to the pedal ganglia and from this early period they are fused to form the pleuro-pedal nerve mass. The cerebro-pedal and cerebro-pleural connectives are formed at the same time by the migration of the nerve cells and fibres to make the anterior nerve ring (fig. 50*b*; fig. 32, Plate 26).

In *Haliotis*, unlike *Patella* (SMITH 1935) and *Paludina* (DRUMMOND 1902), it is impossible to distinguish precisely the limits of the ganglia because the nerve cells spread on to the nerve cords in primitive fashion, as in the Amphineura.

Towards the end of the second day of development the rudiments of the pleuro-visceral cords are brief outgrowths from the pleural ganglia delaminated from the ectoderm of the deepest part of the pallial cavity (figs. 28 and 29, Plate 26; fig. 50c). The process of the right ganglion, the definitive supra-oesophageal, is short, and is at the right side of the oesophagus. The process of the left pleural ganglion, the final infra-oesophageal, is slightly longer and is already ventral to the oesophagus, and its extremity points to the right, towards the ventral part of the pallial cavity, which is on the right side of the body after pelagic 90° torsion. Thus there is already an indication of the influence of the 90° torsion on the nervous system, but it is so far obvious only in the infra-oesophageal visceral process. The ectoderm overlying this process of the left pleural ganglion was on the left side of the body before torsion began, but at the time of delamination it is ventral in position (fig. 50b).

In older veligers the visceral processes consist of nerve cells surrounding nerve fibres (fig. 34, Plate 27). During the 7th and 8th day of development one branchial ganglion, the supra-oesophageal, which in the adult belongs to the topographical left side, is initiated by a collection of nerve cells in the roof of the pallial cavity on the dorsal side of the body (fig. 51d; fig. 35, Plate 27). It innervates the first ctenidial rudiment, which is merely a ciliated mantle ridge at this stage. This branchial ganglion connects with the supra-oesophageal visceral nerve cord when the velum has almost disintegrated, at an age varying from 9 to 14 days (fig. 51e). At this latter time the supra-oesophageal cord is anterior to the sub-oesophageal cord and passes round the right side of the oesophagus to unite with the dorsal branchial ganglion. There is an enlargement of this ganglion to innervate the osphradial rudiment nearby (fig. 36, Plate 27). The supra-oesophageal pleuro-visceral cord now ends at the branchial ganglion (fig. 51e). At its origin from the left pleural ganglion the sub-oesophageal visceral cord is approximated to the posterior end of the larval retractor muscle. It passes, anteriorly to the developing columellar muscle, beneath the oesophagus and terminates on the right side of the pallial cavity (fig. 34, Plate 27). This pleuro-visceral process has now almost post-torsional streptoneury, but there is yet no trace of the rudiment of the final right ctenidium with its branchial ganglion. This, together with the remainder of the pleuro-visceral loop, develops with the post-veliger expansion of the pallial cavity on the right side of the anus and posteriorly (p. 258).

The labial commissure is derived, during the late veliger stage, from ventral processes which grow out from the cerebral ganglia. The buccal ganglia, which also develop before the velum disappears, appear to arise as outgrowths of the labial processes of the cerebral ganglia. They can be seen in the angle between the oesophagus and the radular diverticulum (fig. 33, Plate 27; fig. 51d and f). The nucleated sheath of the buccal ganglia is continuous with that of the cerebral ganglia. They stain more readily

with cotton red or with borax carmine than do the cells of the neighbouring foregut wall.

In this respect *Haliotis* exhibits another primitive feature, for the buccal ganglia of *Acanthochiton* (HAMMERSTEN and RUNNSTRÖM 1925) develop in this manner. In *Patella vulgata*, however, they arise from local thickenings of the foregut wall (SMITH 1935). In *Paludina* (DRUMMOND 1902) all the ganglia arise separately from the ectoderm and are connected secondarily by the growing out of nerve fibres. In *Haliotis*, even in the adult, the ganglia are scarcely distinguishable from the commissures and connectives because all are clothed with nerve cells.

The cephalic tentacles, eyes and statocysts (fig. 44*b*) are innervated by nerves which develop from the cerebral ganglia during the veliger stage (fig. 35, Plate 27; fig. 51). These nerves are described with the sense organs.

Beginning during the 3rd day, the pedal ganglia are gradually prolonged into long posterior and shorter anterior paired processes (fig. 50*c*). The posterior ones lie side by side and finally elongate into cord-like pedal ganglia (fig. 51). The elongation of the pedal nerve ganglia is a primitive condition, which is found in other members of the Archaeogastropoda.

POST-VELIGER DEVELOPMENT

The final phase of metamorphosis begins at the age of about 12 days and is completed at about 2 months. In *Patella vulgata*, however, metamorphosis appears to be completed earlier, during the 3rd week of development (SMITH 1935).

At the beginning of this phase in *Haliotis* the velum has gone and the creeping habit has already been established for some days. The shell is still dome-shaped but, during creeping, it has a list towards the left side and dextral coiling has begun. The larva is now similar to *Trochus* and one might imagine it developing into an adult with a tall spiral shell and a *Helix* method of retraction. Instead, during post-veliger development, it acquires a limpet-like clinging habit and the shell spiral becomes flattened into an ear-shape.

Foot and Operculum

During early post-veliger life the foot becomes extensive and the plantar surface is relatively larger. By the end of the 3rd week after fertilization the larva loses the early plantigrade method of retraction which begins by folding the posterior part of the pedal sole against the anterior part preparatory to withdrawing into the shell and closing its aperture by the operculum, as in *Helix*. At this time retraction is accomplished by the foot clinging to the rock, using the pedal muscles, together with the related columellar muscle, so that the shell aperture is approximated as closely as possible to the rock surface. This function of the columellar muscle probably helps in producing

the flattening of the shell, which is a characteristic feature of the late metamorphosis of *Haliotis* (figs. 46E, F and G).

Although the operculum ceases to grow and to function after the velum is thrown off, it was present in the post-larva 44 days old, when it was still uncalcified. Its disappearance was not observed, but it is probably lost when the shell is pulled closely to the rock by the columellar muscle. In the metamorphosed specimen at 2 months old it was missing.

Sense Organs

The epipodial tentacles arise considerably later than the cephalic tentacles and the development of the characteristic epipodial fringe of sensory structures is confined to the post-veliger stage.

The dorso-lateral protuberance, which is first seen at the time the velum is disintegrating on the right side of the foot, is then the opercular support. Figs. 11 and 14 on Plate 22 show that, in early post-veligers, the first epipodial tentacle arises on this opercular process. It is, therefore, impossible to agree with the statement of BOUTAN (1899) that in *Haliotis* epipodial tentacles are present immediately the larva begins to creep as in *Fissurella*, and that the epipodium has a pallial origin.

During the 3rd week additional tentacles develop on the dorsal enlargements, which are now on both sides of the foot (fig. 20, Plate 24). According to ROBERT (1902) they develop from the anterior end backwards in *Trochus*, but the reverse is true for *Haliotis*.

The epipodial tentacles develop papillae, some of which bear cells with stiff threads similar to those of the cephalic tentacles and, like the latter, these tentacles are pale green in living larvae. In the 44-day-old specimen there were ten tentacles on each side of the epipodium (fig. 48F).

In the larva 23 days old and in that 44 days old there is a large epipodial tubercle posterior to the right eye (fig. 20, Plate 24; fig. 48F). This possesses cilia, which are very active in the living state. A similar process was also seen in *Trochus* by ROBERT, who called it a sensory structure. It is possible that it may produce an outgoing current from the pallial cavity to the right side of the head for the removal of used respiratory water. Water enters under the left side of the shell, owing to a current produced by the single ctenidium of the left side. In the 2 months old larva, in which the pallial cleft and one shell perforation have developed for the exit of water, this organ has merged into the epipodial fringe and the eye peduncle has outgrown it.

Muscles

At the beginning of post-veliger life the veliger head retractor muscle and the columellar muscle are situated on the antero-left and postero-right sides of the visceral hump respectively, and are approximately equal in size (p. 240; fig. 19, Plate 24; fig. 34, Plate 27). The functional significance of the velum retractor muscle as a head

retractor is now lost and it acquires the new function of attaching the developing left margin of the mantle fold to the shell (fig. 46E). As the pallial cavity is gradually displaced to the left side during hypertrophy of the columellar muscle, the velum retractor muscle migrates from the dorsal position shown in fig. 17 on Plate 23 to the extreme left side of the shell (fig. 19, Plate 24; fig. 46E). In the 2 months old larva, as shown in transverse section in fig. 38, Plate 27, it is insignificant in comparison with the columellar muscle.

The columellar muscle gradually increases in size and importance during metamorphosis. In the late veliger it has the same arrangement as the columellar muscle of gastropods with typical dextrally coiled shells, like *Helix*, for it is attached to the shell in the same position as the rudiment of a columella and runs from the right side of the visceral dome under the right margin of the mantle cavity into the foot (fig. 34, Plate 27). After the 2nd week, however, it ceases to retract the cephalo-pedal mass and operculum into the shell and functions during adhesion and creeping. In post-veligers the columella does not develop, but the shell attachment of the columellar muscle migrates anteriorly, so that it approaches the centre of the last whorl of the shell. It occupies an increasingly larger area on the shell (fig. 52). After the 2nd week of development muscle fibres are added rapidly to the columellar muscle so that it becomes a stout central pillar adhering to the pedal musculature. The gradual change in size and position of the columellar muscle is shown in figs. 19 to 22, Plate 24 and fig. 46). SMITH's statement that, in *Patella*, the ability to retract into the shell is lost during metamorphosis because the larval retractor muscle disappears obviously does not apply to *Haliotis*. In this case it is lost because the columellar muscle, which functions in the early veliger as a foot retractor, hypertrophies and its function is highly modified.

The elaborate buccal musculature develops during late metamorphosis from the mesenchyme cells surrounding the foregut (fig. 33, *mus.c.ru.*, Plate 27). In the larva 23 days old the buccal muscles are beginning to link up with the pedal musculature. This connexion is well seen in sections of the 2 months old specimen, in which the most powerful retractor of the odontophore has its origin in the junction of the columellar and foot muscles and its insertion on the posterior end of the odontophore.

Mantle and Shell

At the beginning of the post-veliger metamorphosis the right mantle fold has grown so considerably that, in living specimens, it extends some distance in advance of the shell and it is reflected posteriorly on the right side of the shell umbo (figs. 11 and 13, Plate 22). It has already been shown on p. 242 that this fold is responsible for the foundation of dextral coiling and the tilt of the shell to the left side. During the end of the 2nd week and throughout the 3rd week this fold proliferates shell so rapidly on the right side that it is far in advance of shell growth on the left side, which is now rolled inwards (fig. 14, Plate 22; fig. 46F and G). The posterior right side of

this asymmetrical peristome is attached to the right side of the umbo (figs. 19 and 20, Plate 24). Fig. 52 shows diagrammatically the progressive shell increments, which are seen in the larvae from the beginning of metamorphosis. BOUTAN (1899) described the protrusion of the mantle on the right side of the shell of post-veligers, but he was unaware that the mantle fold is much larger on the right side from an early veliger stage. This is a consequence of the pelagic half of torsion, which leaves the pallial cavity on the right side. The newly added shell is tinged with pink and has radial ridges and a marked growth ridge beyond the smooth veliger shell (fig. 13, Plate 22).

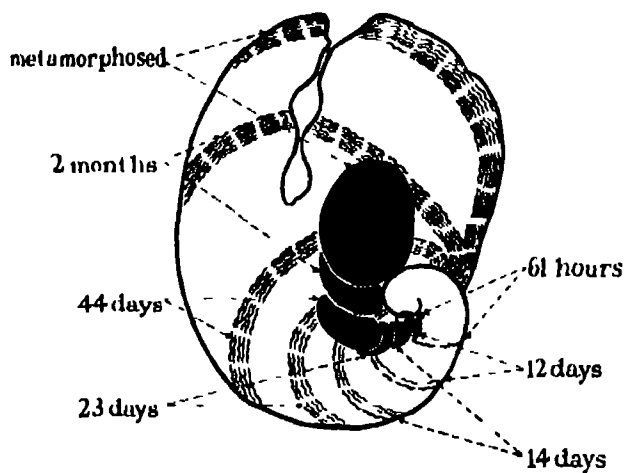


FIG. 52—Diagram to show changes in the shell during metamorphosis. Each pair of converging lines indicates margin of shell and corresponding position of columellar muscle for each stage. Margin of shell is shown by addition of a waved line for increment of shell for each stage. Owing to rapid shell increase on right side, larval shell of 61 hr. lies on its left side in older stages.

In the two larvae which attained 44 days the margin of the shell was still entire, but a small cleft in the mantle on right side of the head had appeared. One of these specimens was fixed, but the other had formed a shell slit corresponding with the mantle cleft, and later, by approximation of the mantle margins, the slit was transformed into the first shell hole, which is shown in the figure of the metamorphosed specimen at 2 months old (fig. 21, Plate 24). At this time the mantle cleft was deeper and had three pallial tentacles.

In the last phase of metamorphosis a mantle pocket develops to cover over the rudiment of the small visceral diverticulum, which borders the right side of the columellar muscle and is shown in fig. 21 on Plate 24. In the adult this is an enlarged conical process accommodating part of the digestive gland and gonad and compensating for the reduced visceral spire (fig. 22, Plate 24).

Post-torsional Displacement of the Pallial Cavity

The rotation of the pallial cavity through 180° is complete by the time the velum is lost and an account of this is given on pp. 243 and 244. From the mid-dorsal position in

the earliest post-veligers (fig. 19, Plate 24; fig. 36, Plate 27), the pallial cavity undergoes a further gradual movement to the left side. Stages in this post-torsional migration are shown in figs. 46 and 48. This displacement is produced by a continuation of differential growth, which appears to be due to hypertrophy of the columellar muscle and its migration towards a central position. Gradual flattening of the shell, which occurs at the same time, may be a contributory cause of the displacement of the pallial cavity (fig. 46). In the metamorphosed specimen 2 months old this cavity is on the extreme left side of the body, which is the position characteristic of the adult (fig. 38, Plate 27; figs. 21 and 22, Plate 24).

Completion of the Pallial Complex

At the close of veliger development the pallial complex comprises the renal organs, the rudiments of one ctenidium, osphradium and hypobranchial gland and the anus, which is situated on the extreme left side of the mid-dorsal pallial cavity (fig. 36, Plate 27).

At the beginning of post-veliger development the pallial cavity has deepened posteriorly and expanded considerably to the left of the anus. In this region is situated the first ctenidial rudiment, which is still a simple ciliated ridge containing the branchial ganglion, overlying a blood space (fig. 19, Plate 24; fig. 36, Plate 27). Immediately ventral to the ctenidial rudiment, on the floor of the mantle cavity, is a corresponding ciliated tegumentary track, which is not, however, a raised band (fig. 36, Plate 27). This may help in maintaining the respiratory current of the deepening pallial cavity. Although the first ctenidial rudiment and its ganglion are now on the left side of the oesophagus (fig. 19, Plate 24), they have taken part only in the last quarter of the 180° torsion of the pallial cavity. In the specimen 23 days old tegumentary ridges have become prominent and form two folds covered with long cilia, which are the rudiments of the ctenidial lamellae. At this time this first formed ctenidium is already on the left side of the body, the displacement of the pallial cavity to the left side having begun. In the specimen 44 days old it has six ctenidial lamellae on either side of the ridge, which now forms the rachis of the ctenidium (fig. 20, Plate 24; fig. 48F). The terminal part bears the osphradium on its proximal border. The topographical left hypobranchial gland lies between the posterior end of the ctenidium and the rectum.

Until the post-veligers are about a month old there is room in the pallial cavity on the right side of the rectum only for the aperture of the right renal organ (fig. 19, Plate 24). In the specimen 44 days old the ciliated band rudiment of the final right ctenidium is present in the now expanding region between the rectum and the columellar muscle (fig. 20, Plate 24). The 2 months old specimen shows much greater expansion of the pallial cavity on the right side of the rectum so that the latter is placed centrally in the cavity. The ctenidium of this side has begun to form lamellae (fig. 46G; fig. 21, Plate 24; fig. 38, Plate 27).

The final right hypobranchial gland also develops when the posterior right side of

the pallial cavity has expanded and at this time the final left one is considerably enlarged. The two glands differ much in size in the adult, but HALLER (1886) denies the existence of the right hypobranchial gland. The ctenidium of this side is also somewhat smaller in the adult than the first formed ctenidium, but both ctenidia are bipectinate, unlike those of *Scissurella*.

The pallial complex in *Halotis* is shown by this investigation to be complete only at the end of 2 months of development.

In the earliest post-veligers the two renal organs are equal in size and their cavities have enlarged. In the specimen 23 days old the one on the right of the rectum is slightly larger than the one on the left (fig. 20, Plate 24), as in the post-larval *Patella* (SMITH 1935). The left one, which has a simple cavity, is a little anterior to the right one. The latter now has its epithelium thrown into a few folds, which become very complicated tubules in the adult (fig. 37, Plate 27). In the adult, the two renal organs are of very unequal size (figs. 21 and 22, Plate 24). The large one on the right is excretory and serves also for passage of the genital products through its cavity and orifice to the pallial cavity.

The heart is first noticed in living early post-veligers by its pulsation in the neighbourhood of the rectum, slightly posterior to the renal organs (fig. 14, Plate 22). It develops during early metamorphosis by ingrowth of the cells of the pericardial rudiment, which it has been shown arises from the solid pericardial rudiment attaching the two renal rudiments (pp. 232 and 246). The ingrowing cells of the pericardial wall become arranged to envelop the rectum and form the ventricle, as in lamellibranchs.

Some of the cells of the heart rudiment connect with the efferent ctenidial vessel of the final left ctenidium and form the definitive left auricle. Fig. 49c, which is part of a transverse section of the 2 months old metamorphosed specimen, shows the left auricle in the region of its ventricular aperture and the anterior part of the right auricle. It is clear from an examination of other sections in this series that the right auricle is about one-quarter the size of the left auricle.

Digestive System

In the early post-veligers the short cylindrical snout, with its vertical mouth, is developed (fig. 48E; fig. 14, Plate 22). The laterally placed chitinous jaws are already forming from the aggregation of perpendicular rods, which are secreted by the specialized columnar cells internal to the buccal opening. The jaws and lips move laterally and the buccal cavity has acquired dorso-lateral ciliated pockets. The spherical rudiment of the odontophore apparatus, which is shown in fig. 19, Plate 24, can be seen, in the transparent living specimens, to move rhythmically in antero-posterior direction. It has already been shown on p. 253 that the main retractor muscle of this apparatus has its origin in the anterior part of the columellar and pedal muscular mass. The rhythmic movements of the odontophore may help the circulation, even at this early stage.

The two dorsal supporting cartilages and the controlling muscles of the odontophore apparatus begin to develop, during the late veliger, as derivatives of the mesoderm in the region ventral to the buccal cavity (fig. 33, *mus.c.ru.*, Plate 27). The odontophore cartilages enlarge as the radula is used more and more, but even in the 2 months old post-larva the extra cartilages, found in the adult, are not developed.

At the end of the first fortnight of development the radula can be protracted and retracted by the movements of the muscular odontophore apparatus. In post-veligers 3 weeks old the distal end of the radula can be used for rasping off fragments of seaweed. Evidence of this is provided by fragments of the more delicate red seaweeds which were found in sections passing through the stomach. At this time there are nine large teeth and five minute marginals on either side of them for every row. The teeth increase in size and, after 2 months of development, there are five lateral teeth on each side of the central tooth and the marginals have increased in number, so that the typical radula of the adult is fully developed (fig. 38, Plate 27). In *Patella* the radula of the adult becomes more specialized than in that of *Haliotis* and the median tooth is lost (SMITH 1935).

In the earliest post-veligers the stomach is larger and has expanded to the left side, so that it now occupies a considerable part of the posterior end of the visceral hump. The ventral digestive gland is pushed somewhat anteriorly by the enlarged stomach and it is still bilobed as in the veliger (fig. 19, Plate 24).

During the 3rd week the digestive gland enlarges. This plastic gland is pushed considerably to the right by development of the stomach and enlargement of the oesophagus near it to form the crop. It is now obvious, from a comparison of figs. 19–22 on Plate 24, that the region of the digestive gland, which lies posterior to the columellar muscle in the earliest post-veliger, forms the reduced visceral coil of the adult. A new diverticulum of the digestive gland, with a separate orifice from the stomach, develops towards the close of metamorphosis, on the dorsal side of the visceral hump. It lies to the right side of the stomach and developing crop and is that lobe of the digestive gland which surrounds the left side of the columellar muscle of the adult (fig. 22, *d.gl.l.l.*, Plate 24).

During the 3rd week there is considerable elongation of the intestine so that another loop forms (fig. 20, Plate 24). An elongated portion of the rectum is suspended from the dorsal wall of the pallial cavity, which has now deepened a great deal posteriorly (figs. 19 and 20, Plate 24; fig. 46). The cilia of the rectum are now very long. While the columellar muscle is developing and the pallial cavity is migrating in the late veligers, the anus gradually travels from the right side of the body to a dorsal position. In the early post-veligers it is mid-dorsal in position (fig. 19, Plate 24) but in the 23-day post-veliger it is already displaced to the dorsal left side by the commencement of hypertrophy of the columellar muscle (fig. 46F). At this time the intestine becomes still more elongated and a loop of it is accommodated near the left margin of the columellar muscle (figs. 20–22, Plate 24).

The small stomach caecum of the adult develops very late and is first seen in a specimen 2.5 mm. long. It is contained in the reduced visceral spire.

Nervous System

Development of the nervous system in the veliger is described on p. 248. Early in post-veliger life the supra-oesophageal pleuro-visceral cord is displaced somewhat to the left side by the enlarging columellar muscle. This cord now passes dorsally to the gut and terminates in the single branchial ganglion, which is now on the left side of the pallial cavity (fig. 51e). At this time the pallial cavity has completed 180° torsion and the proximal halves of the pleuro-visceral cords show the streptoneurous condition, but the typical figure of eight made by these cords in the adult is not yet complete.

In the 23-day post-veliger there is still only one branchial ganglion, but the pleural ganglia have processes, which are the rudiments of the external pallial nerves. These were clearly seen in the reconstruction. Since they develop after 180° torsion is accomplished, the external pallial nerves are uncrossed. Dialyneury must be established later, but it was not discovered in the sections of the 2 months old specimen.

At 44 days after fertilization the rudiment of the second ctenidium, that of the post-torsional right side, is obvious and the branchial ganglion, which innervates it, is initiated from the integument on the extreme right margin of the pallial cavity (fig. 38, Plate 27). Like the left one, it is a branchial and osphradial centre. In the sections of a specimen 2 months old there is now a slender union of the final right branchial ganglion with the infra-oesophageal visceral cord (fig. 51f). The pleuro-visceral cords no longer terminate at the branchial ganglion region, for there are short posterior prolongations immediately beneath the epithelium of the floor of the pallial cavity which unite to form the short visceral ganglion portion of the loop. The lateral branchial ganglia are now unlike typical parietal ganglia, because they are placed on brief off-shoots of the visceral cords. As in the adult, there is little enlargement of the single visceral ganglion. This posterior part of the visceral loop is situated dorsally to most of the digestive system, although that part of the rectum which is attached to the roof of the pallial cavity is bent over dorsally to the visceral ganglion region (fig. 37, Plate 27).

The pedal ganglion cords steadily increase in length as the foot elongates and, in the metamorphosed post-larva, the two posterior cords are connected by a few nerve cells making fine irregular transverse commissures (fig. 51f). In addition to minute branches in the foot there are dorsal strands which innervate the developing epipodium.

The investigation shows that parts of the nervous system of the adult are added gradually throughout the whole period of metamorphosis and the nervous system is completely established only after a developmental period of about 2 months.

DISCUSSION

Asymmetrical Coiling of the Visceral Hump

With the one exception of ANDERSEN (1924), all the more recent views concerning the evolution of the asymmetry of gastropods are concordant in the following hypotheses: symmetrical exogastric coiling of the shell began before the onset of torsion and was independent of it; the pallial cavity was transferred, during torsion, from a posterior into an anterior position, before shell growth began to be asymmetrical; because of the loss of equilibrium when creeping began, the heavy visceral hump fell to one side and pressed upon the foot so that shell growth was retarded on that side and sinistral or dextral rolling resulted. It is presumed by BOUTAN (1899-1919) and by NAEF (1913 and 1926) that in dextral forms the left side of the visceral hump is the heavier and in sinistral forms the lighter, but this remains to be proved.

The conclusions arrived at from the investigations of the development of *Haliotis* appear to throw new light on these suppositions.

In *Haliotis* the pre-torsional shell is symmetrical and has a rudiment of nautiloid coiling before torsion begins; since 90°, only, of rotation occurs in the pelagic period, the shell is then only half endogastric and the pallial cavity is transferred only as far as the right side. With regard to the initiation of dextral coiling in *Haliotis* two factors appear to be responsible.

First, as a consequence of the incompleteness of the early torsional process, the pallial fold remains on the right side of the body for some days and therefore the new part of the shell, which is proliferated from it on this side of the body, is always in advance of that on the left side. The shell thus becomes asymmetrical and such a shell must inevitably lean towards the left side during creeping.

Secondly, it has been shown that, at the time the first phase of torsion has occurred, the main bulk of the visceral mass, comprising the velum retractor muscle and the digestive gland, lies on the left side, whereas the pallial cavity is on the right side. Thus it appears evident that the left side must be heavier.

Consequently the side to which the visceral mass falls during early creeping is determined in *Haliotis* by these two mechanical factors and dextral coiling is the result.

During late metamorphosis the pallial cavity is displaced to the left side by hypertrophy of the columellar muscle, but the addition of new shell is still greater on the right than on the left side, owing to the activity of the extensive right mantle fold.

Theories of Gastropod Torsion

The hypotheses which have been formulated to explain the evolution of the torsion process are numerous. SIMROTH (1896-1907), BOUTAN (1899, 1902, 1919), ROBERT (1902) and DRUMMOND (1902) have summarized the older theories, therefore only the salient points concerned in the recent theories will be mentioned.

Since ANDERSEN (1924) stands alone among the modern theorists, his view may be

stated separately. From his work on the development of *Paludina* he concludes that larval torsion does not bring about streptoneury. He states that the larva develops an untwisted visceral loop, which is gradually twisted into a figure of eight, due entirely to the dextral spiral twist of the visceral hump during late metamorphosis. To accomplish the streptoneury, two and a half spiral windings must be completed. In ANDERSEN's view there is stretching and overgrowth on the right side, and the left side is not inverted, as has been represented by other writers. His description of the nervous system does not, however, agree with that of DRUMMOND (1902). The evidence from the development of *Haliotis* does not help to substantiate his conclusions.

The larvae of *Haliotis*, as of *Patella* (SMITH 1935), do not develop a complete untwisted visceral loop. In this connexion SMITH (p. 116) says of *Patella*, "It is of particular importance to note that, though the pedal and pleural ganglia begin to develop before the torsion process is complete, it is not until afterwards that the visceral and pleural ganglia are formed (delaminated), so that the twist in the visceral loop is not produced during the torsional rotation. It may be that although the visceral ganglia are not formed at this time, yet the ectoderm to which they owe their origin has already become differentiated physiologically." In *Haliotis* the pleuro-visceral loop originates as processes of the pleural ganglia, which are delaminated from the ectoderm of the twisted "neck" region soon after it has been involved in the first half of torsion. The visceral portion of the loop is formed from the ectoderm of the floor of the pallial cavity only at the final phase of metamorphosis. The stretching and overgrowth of the right side of the pallial cavity and its organs, to which ANDERSEN refers, appears to take place in *Haliotis*, not after the formation of the complete pleuro-visceral loop, but during its development.

Other theorists have been greatly influenced by the work of BOUTAN (1886-1919). In his view, the varying degrees to which streptoneury or euthyneury are exhibited depends on the relative amount of foot and shell development in the early veliger. If both organs are well developed at the same time, they interfere with one another and the conflict of pressure brings about torsion and complete streptoneury. Little development of one of these organs results in euthyneurous types, brought about by a process which he calls "larval deviation" rather than by a process of detorsion. Considerable importance has been attached by theorists to his claim to have observed 180° torsion to take place in 2 or 3 min. in *Acmaea* and almost as rapidly in *Haliotis*. MURAYAMA (1935) assumes that 180° is likewise involved in the rapid pelagic rotation of *H. gigantea*. In *Trochus* and in *Patella vulgata*, ROBERT (1902) and SMITH (1935), respectively, state that torsion is completed in the early pelagic rotation.

From the preceding account of the development of *Haliotis tuberculata* it is obvious that BOUTAN's statement is not fully justified for this species. In *Haliotis* a rapid torsion does actually take place in early pelagic life, but it involves only 90° rotation and the further 90° of revolution are completed gradually after many days have elapsed.

From the work of BOUTAN on *Acmaea* (1899) and DRUMMOND (1902) and ERLANGER

(1891) on *Paludina*, NAEF (1913) concluded that slow achievement of torsion by growth processes is a secondary modification found only in less primitive gastropods. There is obvious discrepancy between NAEF's conclusion and the ontogenetic facts for *Haliotis*, for in this undoubtedly primitive member of the Prosobranchia, the accomplishment of complete torsion takes about 2 weeks.

In the light of the existing knowledge of gastropods, the theories of torsion which have been favourably received have sought for the cause of the twist during the adult life of a hypothetical primitive mollusc (BUTSCHLI 1887, LANG 1891, PLATE 1896, FLEURE 1902 and NAEF 1913). Others are of opinion that torsion was not primarily developed in the interest of the adult, but is a larval adaptation; those who seek for ontogenetic evidence are BOUTAN (1899), ROBERT (1902), PELSENEER (1911), GARSTANG (1928). How far ontogenetic facts can be used in relation to phylogenetic theory is problematical. If only from the point of view of their possible bearing on the existing theoretical views, such facts may be enlightening, for as DRUMMOND (1902) remarked, knowledge of the organogenesis of gastropods is so meagre, that a study of the development of even a single type is helpful.

From the embryological point of view it has been shown by CRAMPTON (1894) that asymmetry is foreshadowed in the cleavage of the ovum, since in sinistral forms there is a complete reversal of the cleavage planes as compared with dextral gastropods. Moreover, it has been shown by CONKLIN for *Crepidula* (1897) and by ROBERT for *Trochus* (1902) that there is asymmetry at the time of gastrulation, after which process the right side is slightly larger than the left.

THIELE (1902), in his criticism of BOUTAN's view that torsion results from the conflict of growth of the developing foot and shell, supports the hypothesis that there must be asymmetry in some form before these organs are formed and therefore they can be only a secondary cause of torsion. DRUMMOND (1902) states that unfortunately in *Paludina* torsion begins very early and is taking place at the same time as ano-pedal flexure, so that she can demonstrate only that the liver and the rudiment of the mantle cavity are never completely symmetrical.

The description of *Haliotis* development shows that the two mesoderm bands develop unequally, the rudiment of the shell develops somewhat to the right of the dorsal position and only later extends ventrally and to the left to make an almost bilaterally symmetrical cup. The velum retractor muscle develops on the right side, as in *Patella vulgata* (SMITH 1935) and the "liver" rudiment occupies the left side of the visceral hump. In *Haliotis*, therefore, even more than in *Patella*, there is ample evidence that the veliger is already asymmetrical pre-torsionally.

BOUTAN (1899), NAEF (1913, 1926) and GARSTANG (1928) find it difficult to conceive that a rotation of 180° is accomplished by a growth process alone and imagine that a certain amount of true twisting by muscular contractions must occur. GARSTANG suggests that the only mutation required to start torsion in a hypothetical larval ancestor is the development of asymmetrical retractor muscles. Torsion could be

accomplished by a right-sided cephalic retractor, with a posterior attachment and a left-sided pedal retractor, with a more anterior attachment. It remains, in GARSTANG's view, for further investigation to show if two such muscles, functioning more or less at right angles to one another, do actually exist when torsion begins.

In *Haliotis* (fig. 43; figs. 15 and 15a, Plate 23) as in *Patella* (SMITH 1935, figs. 11a and 11b) the velum retractor muscle develops on the right side. In *Haliotis* there is, moreover, an indication that ancestrally there may have been a pair of retractor muscles, but because of the asymmetrical development of the primitive gut on the pre-torsional left side, the retractor muscle of that side is arrested in development until creeping begins (fig. 25, Plate 25; fig. 44). Then it develops into the columellar muscle. The supposition of SMITH (1935), NAEF (1913, 1926), PELSENEER (1911) and others that the columellar muscle is homologous with the velum retractor muscle is therefore erroneous.

Although the developmental evidence in *Haliotis* lends support to SMITH's view that the right side retractor muscle provides the main mechanical cause for the beginning of torsion, it neither supports his suggestion that this muscle was originally an unpaired dorsal one nor his statement that the complete 180° is involved in the pelagic rotation. It has been shown that 90°, only, is involved in this rapid rotation. The remainder of torsion appears to be caused mainly by the development of the columellar muscle, which is situated on the right side of the pallial cavity. By its gradual enlargement it suppresses the development of the pallial region on that side. With the antero-left migration of the columellar muscle and the velum retractor muscle the pallial region gradually expands and moves dorsally, so that the second phase of torsion is completed. The second half of torsion is, therefore, brought about by differential growth, which is an inevitable result of the first phase of torsion, after which the pallial cavity was situated on the right side of the body.

In addition to the 180° torsion, which is brought about during the first 2 weeks of development, continued migration of the pallial cavity to the left side takes place during the late development of *Haliotis*. This is due to hypertrophy and further migration of the columellar muscle and thus the spatial relations peculiar to the adult are produced.

It might be suggested with caution that, in opisthobranchs with a very reduced columellar muscle, the cause for completion of torsion is removed, but it is irrelevant here to discuss the probability of deviation or of detorsion in these forms.

Order of Formation of the Pallial Organs

The interpretations of the phenomenon of gastropod torsion have been based on the assumption that the pallial complex of the hypothetical ancestor is already constituted before torsion begins and that it is simply inverted during the process. In *Haliotis* and in *Patella* (SMITH 1935) this is not the case. In both genera the protodaeum and the solid mesoderm rudiments of the kidneys are the only representatives of the pallial

complex when torsion begins. Even in *Paludina*, in which the developmental stages are abbreviated owing to viviparity, the single ctenidium and the visceral part of the pleuro-visceral loop do not develop until the late larval stage, after torsion is complete (DRUMMOND 1902).

During the second phase of torsion in *Haliotis* the first ctenidial rudiment, the definitive left, together with its branchial ganglion migrates with the antero-left migration of the pallial roof. The major portion of the pallial cavity, on the definitive left side of the rectum, grows and expands in a dorsal direction, but the topographical right region of the cavity does not begin to develop until after torsion is complete. Until this part of the pallial cavity enlarges there is no room for the topographical right ctenidium, which therefore arises in the last stage of development.

The zygobranchiate members of the Archaeogastropoda have the ctenidia placed on either side of the anal complex. These forms have in common a characteristic system of currents maintained by cilia. Fresh supplies of water pass to the ctenidia from around the shell margin; deoxygenated water and products of excretion invariably escape from the middle of the pallial cavity through a slit, a hole or series of holes, placed dorsally to the anal complex. GARSTANG (1928) explained that the palaeontological evidence in *Bellerophon* and the shell evidence in the recent forms *Pleurotomaria* and *Fissurella* show that the slit or hole never develops until the end of metamorphosis; thus *Fissurella* has added the metamorphosing young adult stage of *Emarginula* to its ontogeny. GARSTANG points out that this theory differs slightly from the Haeckelian hypothesis, according to which *Fissurella* and *Haliotis* would inherit all that the adult *Emarginula* had developed. In GARSTANG's view the time of divergence is different for different organs.

GARSTANG further suggests that since the shell outlet corresponds in position with the anus and renal openings, it is probably made by inhibition of mantle and consequently of shell growth, due to the stream of poisoned water. The mantle can only grow together again when out of the range of this current. In *Haliotis* the outgoing current is vertical and there is alternate splitting and closing of the mantle margin as the pallial cavity grows forwards. The description of the development of *Haliotis* shows that the first shell hole actually does appear at the close of metamorphosis and, moreover, the final right ctenidium develops only at about the same time. The "sanitary" arrangements during the intervening period after the formation of the first ctenidium may be presumed to resemble those of the members of the Archaeogastropoda, which have a single ctenidium supplied by fresh water from under the left shell margin. After use, the water passes away above the anus on the right side of the pallial cavity.

The fact that the topographical right ctenidium and the hypobranchial gland of that side, which secretes mucus to clean the respiratory chamber, do not appear until a month later than those organs of the definitive left side appears to be significant. Both ctenidia in *Haliotis* are bipectinate, whereas in *Scissurella* the definitive left one is monopectinate; in the adult *Haliotis*, as in *Pleurotomaria* and *Scissurella*, the definitive

right ctenidium is invariably somewhat smaller than the left one, and this difference in size also applies to the hypobranchial glands.

Two equal-sized ctenidia are never present in prosobranchs with asymmetrically coiled shells. Since the gills are the latest organs to form in gastropods, it would be anticipated that the asymmetry would affect their development more than that of other organs. It is a generally accepted hypothesis that asymmetrical coiling of the shell and enclosed visceral mass produces pressure on one side of the pallial cavity, as a result of which the organs of this side are reduced or atrophied. This is substantiated by the order of development of the pallial organs of *Haliotis*. It is important to note that the situation of the pallial cavity, on the right side of the body for some days after the first phase of torsion, is the cause both of dextral coiling and of compression of the right side of the cavity.

The delayed development of the pallial organs of the right side in *Haliotis* foreshadows the complete disappearance of the organs of that side in forms like *Acmaea* and *Trochus* of the Archaeogastropoda and in the Mesogastropoda.

SUMMARY

1. The development of *Haliotis*, which takes about 2 months, is described from living specimens, serial sections and reconstructions. Comparison is drawn with the development of *Patella*, *Trochus* and *Paludina*.

2. The trochophore has neither apical cilia nor a telotroch, but the veliger has transitory apical cilia. The larvae are mainly benthic after 2 days, although the velum persists for 2 weeks.

3. The formation of mesoderm resembles that process in *Patella*, but the mesoderm band of the right side is larger than that of the left side. Pre-torsionally the velum retractor muscle arises from this mesoderm band and is asymmetrically placed.

4. Asymmetry is also shown in the rudiments of the molluscan organs.

5. The asymmetrical velum retractor muscle is mainly responsible for the rapid first 90° of torsion. The rudiment of the operculum develops immediately before torsion begins and is probably a contributory cause of the beginning of rotation. BOUTAN and MURAYAMA are shown to be mistaken in the hypothesis that 180° is involved in this pelagic rotation.

6. The second half of torsion of the pallial region is brought about slowly, by differential growth. The development and migration of the columellar muscle is mainly responsible for it.

7. Contrary to the general supposition concerning gastropod veligers, the velum retractor muscle does not become the columellar muscle. The latter develops from mesoderm cells of the pre-torsional left side, which are arrested in development until the early plantigrade stage. At the time the velum is lost, the two muscles are equal in size and bilaterally situated. During late metamorphosis the columellar muscle

hypertrophies and migrates to a central shell attachment, but the vestige of the velum retractor is an insignificant muscle on the left side.

8. After the first half of torsion, the pallial cavity remains on the right side for some days. Consequently the visceral mass is heavier on the left side and the pallial fold is responsible for rapid addition to the shell on the right side. The shell, therefore, leans to the left and dextral coiling begins.

9. Pre-torsionally the proctodaeum is the only representative of the pallial complex. The solid rudiments of the renal organs acquire cavities, reno-pericardial canals and renal apertures during the second phase of torsion. They become asymmetrical only during late metamorphosis.

10. The two ctenidia and the two hypobranchial glands develop at widely separated periods and, at the end of development, those of the topographical right side are smaller than those of the left side.

11. The definitive left branchial ganglion connects with the supra-oesophageal process of the right pleural ganglion in the veliger. The definitive right branchial ganglion connects with the infra-oesophageal process of the left pleural ganglion during late metamorphosis. The visceral ganglion region of the pleuro-visceral loop then develops. Its late development may account for its position in relation to the rectum differing from that of the Amphineura.

12. The external pallial nerves arise after the streptoneurous condition is established and are therefore untwisted.

13. Development of the sense organs is traced.

14. The digestive organs arise similarly to those of *Patella*, but the radula retains a more primitive character after metamorphosis.

15. The characters peculiar to *Haliotis* develop in the late plantigrade larva. Hypertrophy of the columellar muscle is accompanied by displacement of the pallial cavity to the left side and shell flattening. The ability to retract into the shell ceases, but the operculum does not fall off for some time after its function is lost. The cleft in the roof of the pallial fold is responsible for the formation of one shell perforation, at the close of metamorphosis. It provides for the increased respiration made possible by the addition of the second ctenidium.

16. The possible bearing of the ontogenetic facts upon phylogenetic theories is discussed.

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KEY TO LETTERING ON FIGURES

<i>a.ao.</i>	anterior aorta.	<i>br g.r.</i>	post-torsional right branchial ganglion.
<i>an.</i>	anus.	<i>buc.cav.d.f.</i>	dorsal fold of buccal cavity.
<i>an.c.</i>	anal cell; position of anus.	<i>buc.g.</i>	buccal ganglion.
<i>ap.c.</i>	apical cell.	<i>c.c.v.</i>	cells cutting off velum.
<i>ap.cil.</i>	apical cilia.	<i>c.mus.</i>	columella muscle.
<i>arch.</i>	archenteron.	<i>c.mus.ru.</i>	rudiment of columellar muscle.
<i>aur l.</i>	left auricle.	<i>ceph.t.</i>	cephalic tentacle.
<i>aur.r.</i>	right auricle.	<i>ceph.t.n.</i>	cephalic tentacle nerve.
<i>bl.</i>	blastopore region.	<i>ceph.t.p.</i>	cephalic tentacle papilla.
<i>br.g.l.</i>	post-torsional left branchial ganglion.	<i>cer.g.</i>	cerebral ganglion.

<i>cer.g.b.</i>	cerebral ganglion band.	<i>mo.</i>	mouth.
<i>cer.g.ru.</i>	cerebral ganglion rudiment.	<i>mus.c.</i>	muscle cell.
<i>cer.ped.c.</i>	cerebro-pedal connective.	<i>mus.c.ru.</i>	rudiment of muscle cell.
<i>cer.pl.c.</i>	cerebro-pleural connective.	<i>o.n.</i>	optic nerve.
<i>chi.</i>	chitinous lining of stomach.	<i>od.ap.</i>	odontophore apparatus.
<i>ct.l.</i>	ctenidium of post-torsional left side.	<i>od.cart.</i>	odontophore cartilages.
<i>ct.l.p.</i>	posterior end of left ctenidium.	<i>oes.</i>	oesophagus.
<i>ct.l.ru.</i>	rudiment of post-torsional left ctenidium.	<i>op.</i>	operculum.
<i>ct.os.ru.l.</i>	ctenidial and osphradial rudiment of post-torsional left side.	<i>op.ru.</i>	rudiment of operculum.
<i>ct.r.</i>	ctenidium of post-torsional right side.	<i>os.</i>	osphradium.
<i>ct.r.ru.</i>	rudiment of post-torsional right ctenidium.	<i>pal.c.</i>	pallial cavity.
<i>d.gl.</i>	digestive gland.	<i>pal.c.r.p.</i>	right posterior horn of pallial cavity.
<i>d.gl.l.</i>	digestive gland lobe.	<i>pal.c.t.</i>	termination of pallial cavity.
<i>d.gl.l.l.</i>	left digestive gland lobe.	<i>pal.cl</i>	pallial cleft.
<i>d.gl.l.r.</i>	right digestive gland lobe.	<i>pal n.r.e.</i>	right external pallial nerve.
<i>d.gl.stom.</i>	opening of digestive gland from larval stomach.	<i>pal.t.</i>	pallial tentacle.
<i>e</i>	eye.	<i>ped.ci.</i>	pedal cilia.
<i>e.l.</i>	left eye.	<i>ped.ep.m.c.</i>	mucous cell of pedal epithelium.
<i>end.</i>	endoderm.	<i>ped.g.</i>	pedal ganglion.
<i>ep.</i>	epipodium.	<i>ped.g.c.</i>	pedal ganglion cord.
<i>ep.la.p.</i>	larval process of epipodium.	<i>ped g l.</i>	left pedal ganglion.
<i>ep.t.</i>	epipodial tentacle.	<i>ped.g r.</i>	right pedal ganglion.
<i>f.g.</i>	foregut.	<i>ped.gl.</i>	pedal gland.
<i>fo.</i>	foot.	<i>ped.ru.</i>	pedal rudiment.
<i>h.ru.</i>	heart rudiment.	<i>ped.s.</i>	pedal sole.
<i>hy.gl.</i>	hypobranchial gland.	<i>per.</i>	pericardium.
<i>in.</i>	intestine.	<i>per.c.</i>	pericardial cavity.
<i>integ.v.c.</i>	integument cutting off velar cell.	<i>per.ru.</i>	rudiment of pericardium.
<i>lab.com.</i>	labial commissure.	<i>pl g.l.</i>	left pleural ganglion.
<i>m.</i>	mouth.	<i>pl g.r</i>	right pleural ganglion.
<i>m.g.</i>	midgut.	<i>pl.ped g.</i>	pleuro-pedal ganglion mass.
<i>m.g.c.</i>	cavity of midgut.	<i>pl.ped g.l.</i>	left pleuro-pedal ganglion mass.
<i>mant.f.</i>	mantle fold.	<i>pl.ped.g.r.</i>	right pleuro-pedal ganglion mass.
<i>mant.f.t.</i>	posterior border of mantle fold.	<i>pr.mes</i>	primitive mesoderm cells.
<i>mant.m.</i>	margin of mantle.	<i>pr.vel.a</i>	pre-velar area.
<i>mant.ru.</i>	mantle rudiment.	<i>pr.vel.c.</i>	pre-velar cell.
<i>mesen.c.</i>	mesenchyme cell.	<i>pr.vel.gr.</i>	groove in pre-velar area.
<i>mes.b.</i>	mesoderm band.	<i>proc.</i>	proctodaeum.
<i>mes.b.u.</i>	ventral union of mesoderm bands.	<i>prot.</i>	prototroch.
<i>mes.ru.</i>	rudiments of mesoderm.	<i>rad.</i>	radula.
<i>mi.f.</i>	mitotic figure.	<i>rad.c.</i>	radular caecum.
		<i>rad.t.</i>	tooth of radula.
		<i>rect.</i>	rectum.
		<i>rect.pr.</i>	primitive rectum.
		<i>ren.ap.l.</i>	post-torsional left renal aperture.
		<i>ren.ap.pr.</i>	precocious renal aperture.

<i>ren.d.ru.</i>	dorsal rudiment of renal organ.	<i>sub.o.pl.v.p.</i>	sub-oesophageal pleuro-visceral process.
<i>ren.l.</i>	post-torsional left renal organ.	<i>sub.o.pl.ru.</i>	rudiment of sub-oesophageal pleuro-visceral process.
<i>ren.per.c.</i>	reno-pericardial canal.	<i>sup.o.pl.v.c.</i>	supra-oesophageal pleuro-visceral cord.
<i>ren.r.</i>	post-torsional right renal organ.	<i>sup.o.pl.v.ru.</i>	rudiment of supra-oesophageal pleuro-visceral cord.
<i>ren.v.</i>	ventral renal organ.	<i>um.</i>	umbo.
<i>ren.v.ru.</i>	ventral rudiment of renal organ.	<i>v.</i>	velum.
<i>sh.</i>	shell.	<i>v.c.</i>	velar cell.
<i>sh.at.integ.</i>	shell attachment of integument.	<i>v.c.n.</i>	nucleus of velar cell.
<i>sh.gl.</i>	shell gland.	<i>v.r.mus.</i>	velum retractor muscle.
<i>sh.m.</i>	margin of shell.	<i>v.r.mus.ped.</i>	pedal addition to velum retractor muscle.
<i>sh.p.</i>	shell perforation.	<i>v.r.mus.ves.</i>	vestige of velum retractor muscle.
<i>sh.ru.</i>	shell rudiment.	<i>v.v.</i>	vestige of velum.
<i>sn.</i>	snout.	<i>ven.</i>	ventricle.
<i>st.</i>	stomodaeum.	<i>visc.</i>	reduced visceral coil.
<i>stat.</i>	statocyst.	<i>visc.g.</i>	visceral ganglion.
<i>stat.in.</i>	statocyst invagination.		
<i>stat.n.</i>	statocyst nerve.		
<i>stom.</i>	stomach.		
<i>sub.o.pl.v.c.</i>	sub-oesophageal pleuro-visceral cord.		

DESCRIPTION OF PLATES

PLATE 21

Drawings of living veligers up to the time when pelagic life ceases. The details of the muscles were added after determination of their arrangement from reconstructions based on serial sections. The orientation of the larva is indicated by letters. $\times 170$ linear approx.

FIG. 1—Right side of veliger at 27 hr. after fertilization, a short time before torsion begins. The ventral pallial cavity and the velum retractor muscle can be seen on the right side.

FIG. 2—Left side of veliger 31½ hr. after fertilization. The rudiment of the operculum is present, although inconspicuous.

FIG. 3—Ventral view of veliger 33 hr. old. Torsion has begun and the visceral hump has rotated, so that the pallial cavity is displaced somewhat to the right side of the foot. The cephalo-pedal mass is partially retracted into the shell, but the operculum does not completely close the shell until several hours later.

FIG. 4—Left side view of veliger 35 hr. old. 90° of torsion have taken place, so that the shell is half endogastric. This view shows the "neck" region, which is involved in the twisting.

FIG. 5—Veliger 54 hr. old seen from the left side. At this time the larvae swim for short periods only. The rudiments of the sense organs are obvious.

FIG. 6—View of left side of veliger at 79 hr. after fertilization. The larvae are now entirely benthic, although creeping is not successfully accomplished until about 24 hr. later. The velum retractor muscle has migrated to the left side. The shell is endogastric, although the pallial cavity is still on the right side of the body.

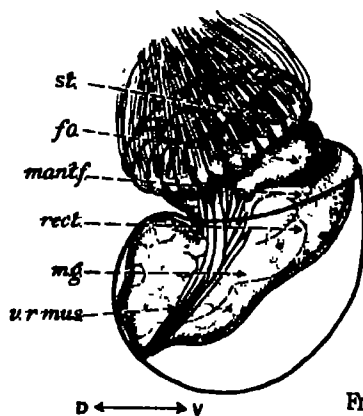


FIG 1

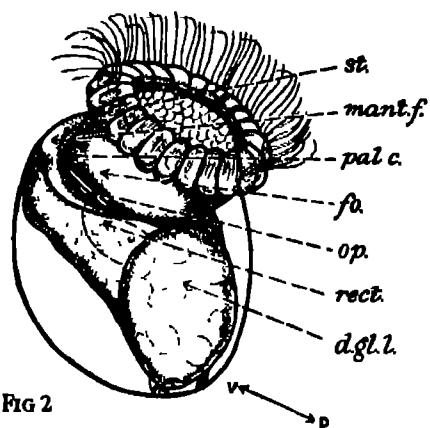


FIG 2

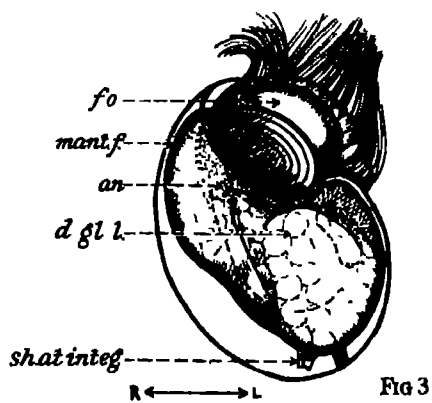


FIG 3

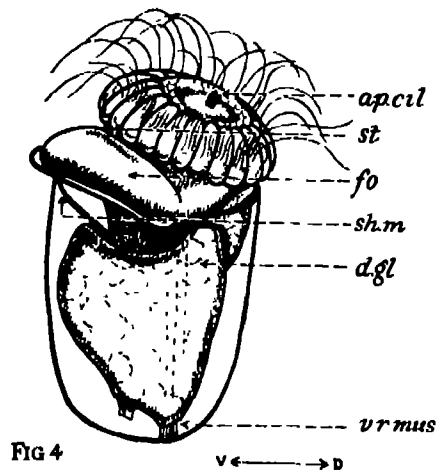


FIG 4

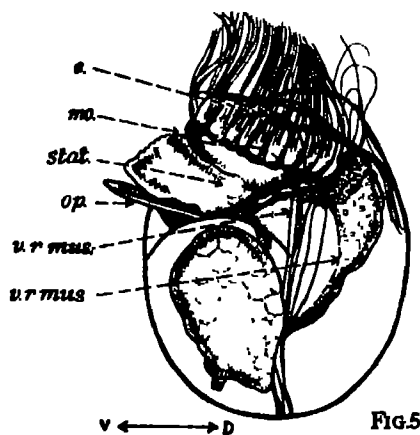


FIG 5

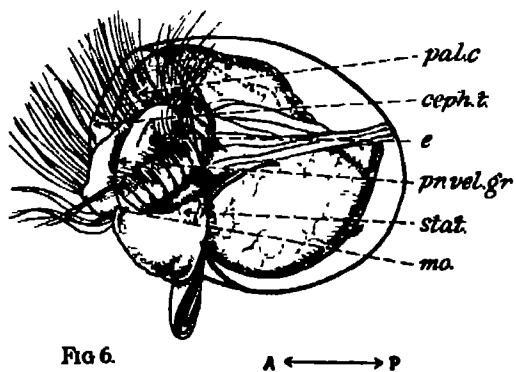


FIG 6.

PLATE 22

Drawings of living larvae from the beginning of benthic life. The figures of the younger stages show the larvae making attempts at creeping. In the later stages the gradual loss of velum is shown. The areas of attachment of muscles are labelled as the muscles themselves.

× 160 linear approx.

FIG. 7—Larva 5 days old, seen from the left side. The operculum is in process of releasing the head and ciliated pedal sole from the shell.

FIG. 8—Larva 6 days old, seen from the right side. The specimen was about to cling by means of the pedal sole. Note the opercular process of the metapodium and the beginnings of asymmetrical growth of the shell on the right side. The renal organ rudiments and the developing columellar muscle on the right side are shown. The eyes are now pedunculated.

FIG. 9—Left side view of larva 4½ days old, which was clinging with the pedal sole and appeared to make contortions of the “neck” region in order to lift the shell and its contents into a comfortable position.

FIG. 10—Dorsal view of larva 10 days old, a few hours after the velum was thrown off. The pallial cavity is still somewhat to the right side of the body. The area of attachment of the velum retractor muscle is now near the left side of the shell.

FIG. 11—Right side view of post-veliger 11 days after fertilization. A few hours previously the remainder of the velum was thrown off. The mantle fold projects beyond the shell margin and the beginning of asymmetrical growth of the shell is obvious on this side. The pallial cavity has reached an almost dorsal position, but the anus is still at the extreme right side of it.

FIG. 12—Left side view of post-veliger 11 days old. This was drawn from the same specimen as the above, at a time when it was emerging from the shell. The area of fixation of the velum retractor muscle is now near the left margin of the shell.

FIG. 13—Post-veliger 12 days old, seen from the anterior right side. The pallial cavity is now dorsal. The shell is markedly asymmetrical and leaning towards the left side. The first ctenidial and osphradial rudiment, the definitive left, is seen in dorsal position. Ciliary movement made this obvious from about the 7th day of development. The radular apparatus moved up and down rhythmically and the jaws were seen to move.

FIG. 14—Ventral view of post-veliger 12 days old, drawn when it was creeping upside down and suspended from the water surface. Asymmetry of the shell and mantle are obvious. As the post-veliger no longer retracts into the shell, the operculum has now lost its function. The first epipodial tentacle, which originates from the opercular process, is seen on the right side.

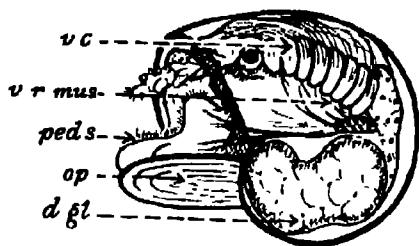


FIG 7

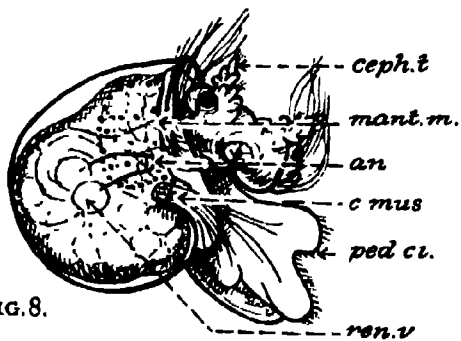


FIG.8.

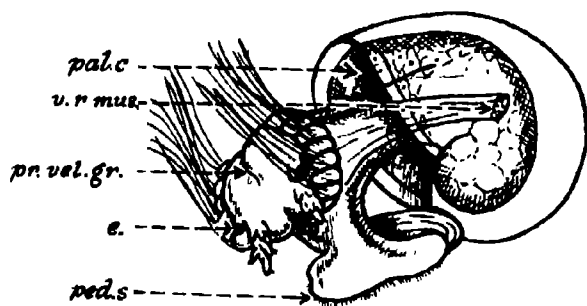


FIG 9

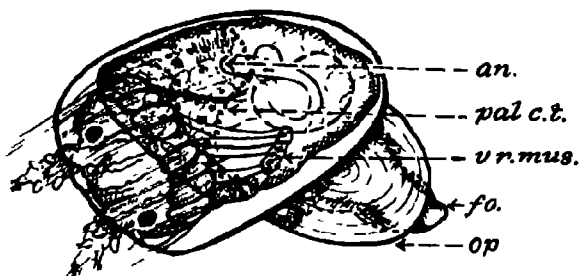


FIG 10.

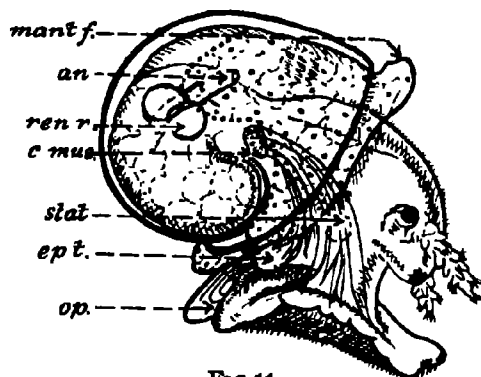


FIG 11.

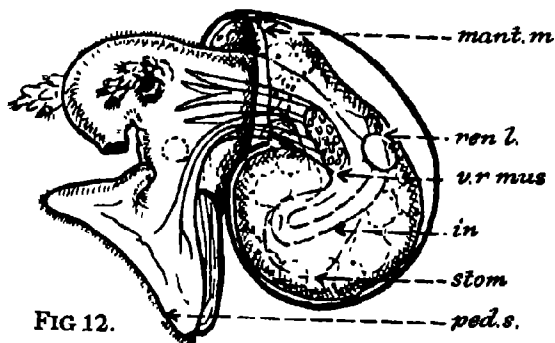


FIG 12.

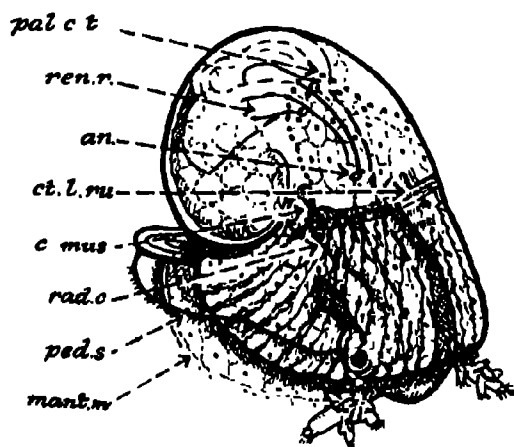


FIG.13.

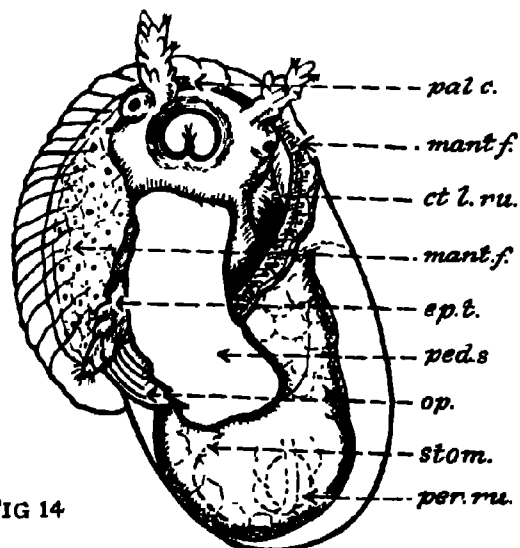


FIG 14

PLATES 23 AND 24

A series of diagrams based on reconstructions from serial sections and on observation of living specimens. The migration of the pallial cavity, the changes in the spatial relations of the velum retractor muscle and columellar muscle and in the digestive system are shown. The shell is indicated by a heavy outline. In Plate 23 the magnification is approximately 200, but in Plate 24 it is indicated for each figure.

PLATE 23

FIG. 15—Dorsal view of veliger immediately before torsion, about 30 hr. old. The pallial cavity is ventral and the retractor muscle curves round the right side.

FIG. 15*a*—Ventral view of the same stage.

FIG. 16—Dorsal view of veliger at about 34 hr. old, soon after rapid torsion of 90° has occurred. The velum retractor muscle is now straight and in dorsal position; the pallial cavity and the rectum are on the right side.

FIG. 16*a*—Ventral view of the same stage.

FIG. 17—Dorsal view of retracted veliger 61 hr. old. The pallial cavity and the anus remain on the right side and the renal rudiments are dorsal and ventral to the rectum. The rudiment of the columellar muscle is seen on the right side.

FIG. 18—Dorsal view of larva 14 days old. This was the oldest specimen which retained vestiges of the velum. The shell is asymmetrical, the pallial cavity has migrated almost into dorsal position and the mantle fold has extended round the left side. The first ctenidial rudiment, the definitive left, is dorsal and the velum retractor muscle has migrated to the left side.

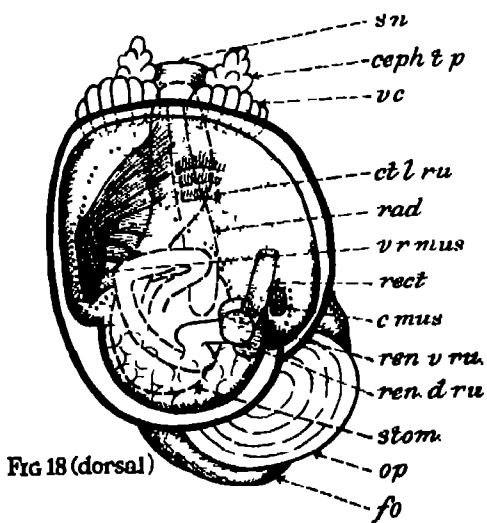
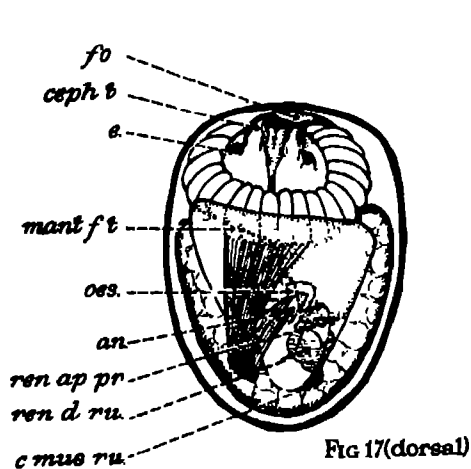
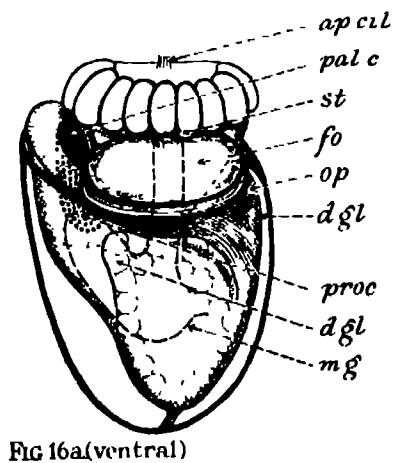
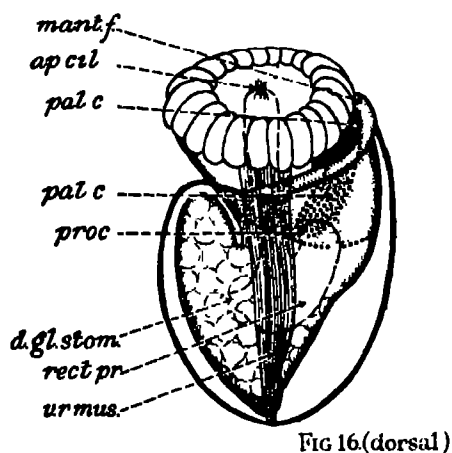
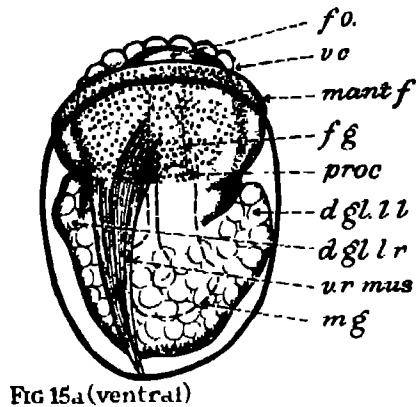
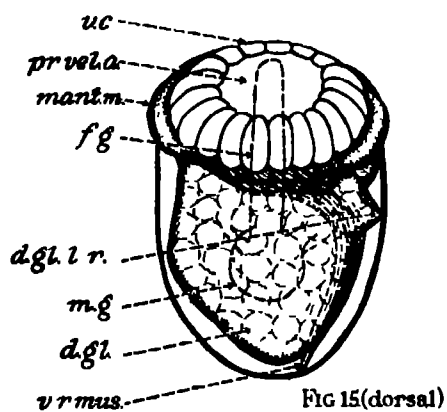


PLATE 24

FIG. 19—Post-veliger 12 days old. This was a specimen which had recently lost the velum. Note the extensive mantle fold of the right side and the dextral coiling of the shell. The pallial cavity has now completed 180° migration from its position before torsion began. The anus still occupies the extreme right side of the pallial cavity and the one ctenidial rudiment, the definitive left, is already slightly to the left side. The two muscles are now about equal in size. $\times 200$ approx.

FIG. 20—Post-veliger 44 days after fertilization. There is one well-developed ctenidium, the final left, and the anus is still placed at the right side of the pallial cavity. The latter is already displaced somewhat to the left side by hypertrophy of the columellar muscle. The pallial cleft has begun to form and the renal organs are now unequal in size. $\times 38$.

FIG. 21—Metamorphosed *Haliotis* 2 months old. There are two ctenidia but the definitive right one is yet very immature. It has begun to form ctenidial lamellae, but is very small in comparison with the definitive right ctenidium, which has its free tip bent over in the diagram. The anus is now in the centre of the pallial cavity, with ctenidia and renal apertures placed in bilaterally symmetrical fashion. Pallial tentacles and one shell hole are shown. $\times 28$ approx.

FIG. 22—Dorsal view of adult *Haliotis* without the shell. The topographical right ctenidium is now only slightly smaller than the topographical left one. The visceral coil is vestigial in comparison with that at 2 months old. The conical process of the digestive gland and gonad, which curves round the columellar muscle into a pocket of the mantle on the right side, has developed from the rudiment seen under the visceral coil in fig. 21. $\times \frac{1}{3}$ approx.

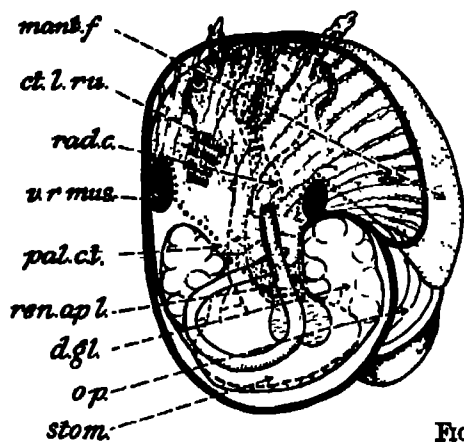


FIG 19

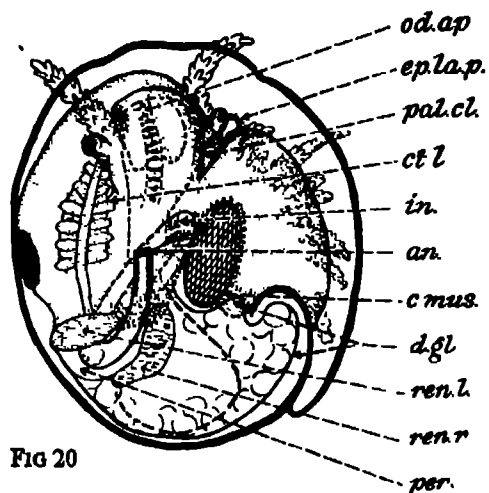


FIG 20

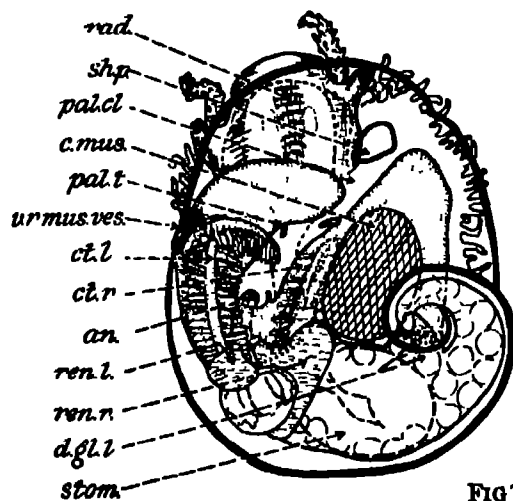


FIG 21

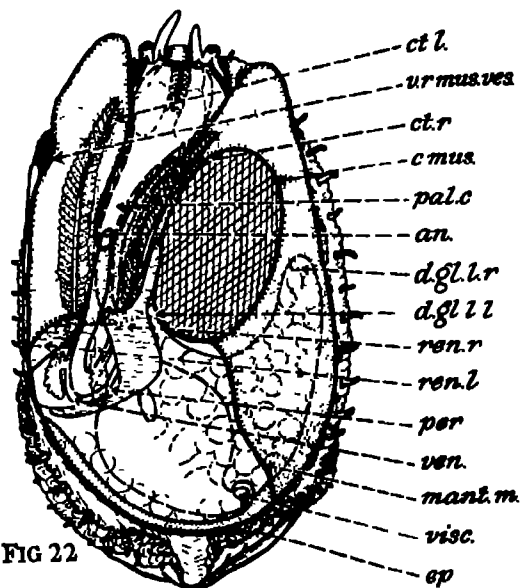


FIG 22

PLATE 25

Sections showing the development of the velum retractor muscle, etc. $\times 450$ approx.

FIG. 23—Transverse section of veliger 19 hr. old, passing through the foot and posterior end of the velum in the neighbourhood of the stomodaeum. The mesoderm bands and the right anterior extremity of the mantle rudiment are seen.

FIG. 24—Parasagittal section of veliger 21 hr. old. The section passes through the developing muscle cells of the right side which, a few hours later, constitute the velum retractor muscle. The mantle fold and foot rudiments are seen.

FIG. 25—Transverse section through the posterior part of the visceral hump of veliger 33 hr. old. In this specimen torsion has not begun. The section shows the cells of the velum retractor muscle situated on the right side of the visceral hump. The two cells on the left side later give rise to the columellar muscle, which is delayed in development until creeping begins.

FIG. 26—Transverse section of veliger 30 hr. old, which is more advanced in development than the specimen shown in fig. 25 and already shows 90° torsion. The section passes through the posterior end of the foot and shows the dorsal position of the velum retractor muscle.

FIG. 27—Frontal section through a veliger of the same age as in fig. 26 with 90° torsion. The section is dorsal to the larval stomach and shows the full length of the cells of the velum retractor muscle, which is now in dorsal position.

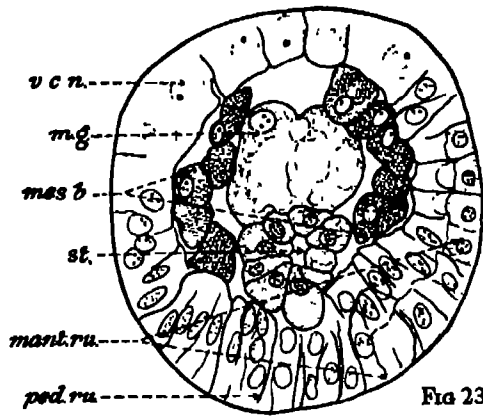


FIG 23

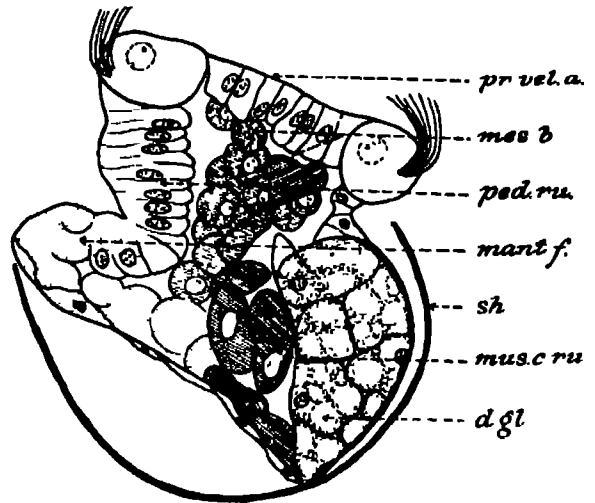


FIG 24

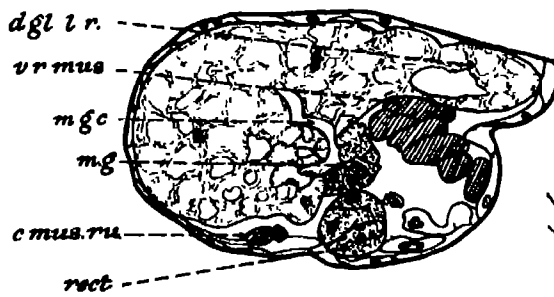


FIG 25

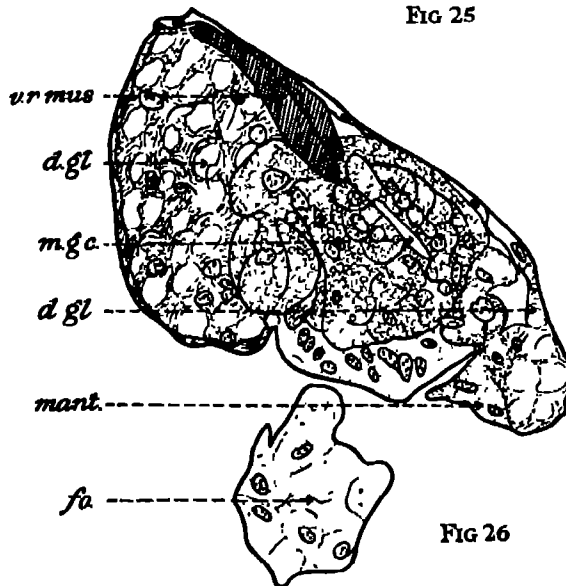


FIG 26

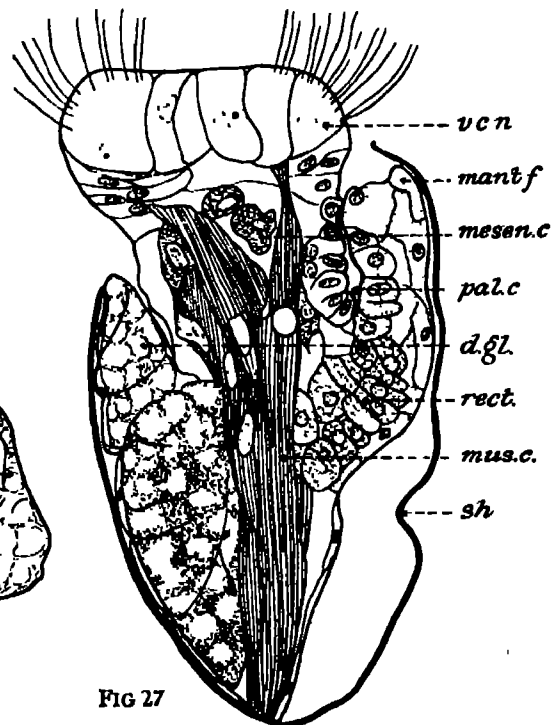


FIG 27

PLATE 26

Sections showing the early stages in development of the nervous system. $\times 450$ approx.

FIGS. 28, 29 and 30—Frontal sections of the same series as in fig. 27 of Plate 25.

FIG. 28—Section next ventrally to that of fig. 27. It passes through the short rudiment of the supra-oesophageal pleuro-visceral process, which is seen on the right side. The pallial cavity, with the rectum, is also on the right side.

FIG. 29—Section ventral to the oesophagus and anal cell, passing through the rudiment of the sub-oesophageal pleuro-visceral process.

FIG. 30—Section passing through the foot, showing the anterior end of the statocyst invagination, rudiments of the cerebral ganglia and cerebro-pedal connective. The insignificant ciliated apical cells are shown.

FIG. 31—Transverse section of veliger 33 hr. old immediately before torsion begins. The section passes through the cephalo-pedal mass and shows the first rudiments of the operculum above the ventral mantle fold. The cerebral and pleural ganglia are almost completely delaminated from the epithelium.

FIG. 32—Transverse section of veliger 61 hr. old, passing through the foot and velum close to the pre-velar plate. On the right side the apical plate cells neighbouring the eye are seen. Note the cerebral ganglion band, pleuro-pedal mass, cerebro-pleural connective, statocysts and operculum.

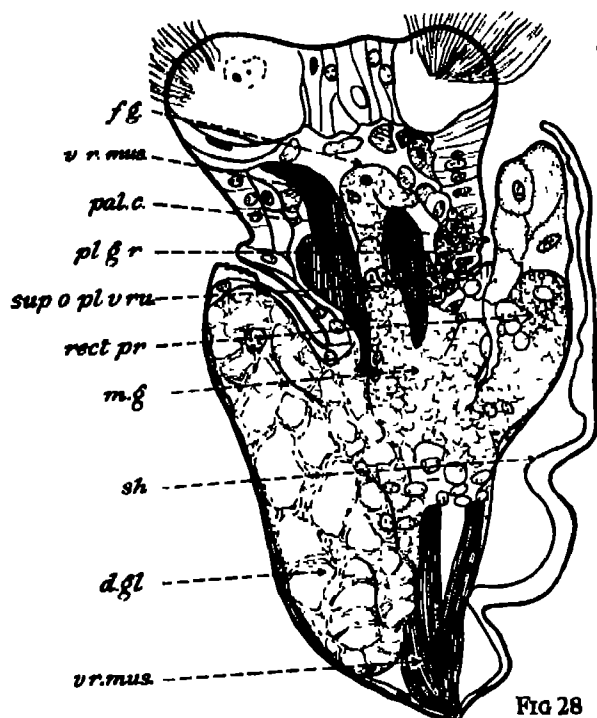


FIG 28

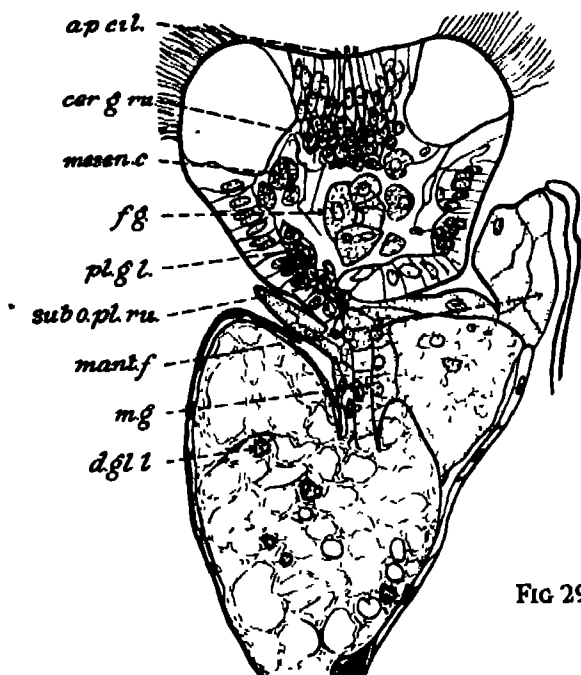


FIG 29

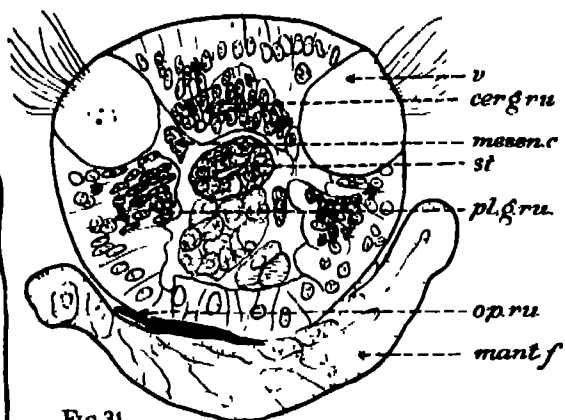


FIG 31

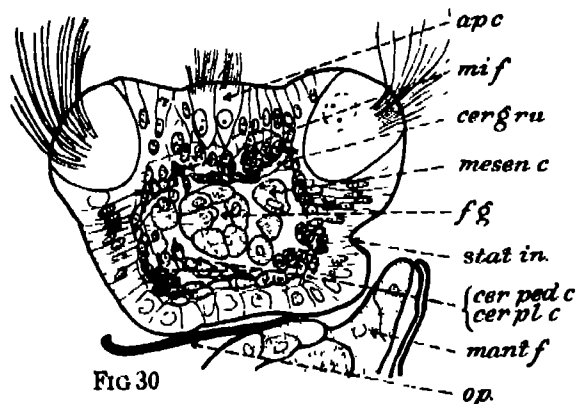


FIG 30

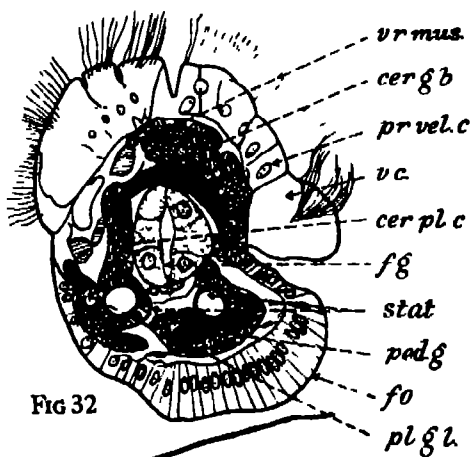


FIG 32

PLATE 27

A series of sections to show the later development of the nervous system, the muscles and the pallial complex. The areas of insertion of the muscles are labelled as the muscles themselves.

FIGS. 33 and 34—Slightly oblique transverse sections passing through the middle region of larva 14 days old. This was the oldest specimen possessing vestiges of the velum; it was somewhat contracted. The section of fig. 33 passes through the anus and the extreme anterior end of the operculum. The pallial cavity is still somewhat to the right of the dorsal position. The supra-oesophageal pleuro-visceral process is seen. In fig. 34 the section passes through the velum retractor and the columellar muscle, which are now almost equal in size. The sub-oesophageal pleuro-visceral process terminates on the right side. $\times 450$ approx.

FIG. 35—Parasagittal section, passing about 15μ to the left side of the median plane and radular caecum, of veliger 7 days old. It passes through the extreme left side of the first ctenidial rudiment (final left). $\times 450$ approx.

FIG. 36—Transverse section through post-veliger 12 days old, the youngest specimen without the velum. The section is slightly anterior to the middle region and passes through the pallial cavity, which is now dorsal, after completing 180° torsion. The rectum is still to the extreme right side of the pallial cavity and there is only one ctenidial rudiment, the topographical left. Parts of the supra- and sub-oesophageal pleuro-visceral processes are seen. $\times 450$ approx.

FIG. 37—Transverse section of metamorphosed *Halotis* 2 mm. long, passing through the viscera ganglion and the posterior end of the pallial cavity, which has two diverticulæ in this region. The two renal organs now differ much in size and appearance as in the adult. $\times 20$ approx.

FIG. 38—Transverse section through the same specimen as in fig. 37, passing through the well-developed definitive left ctenidium and the rudimentary right ctenidium. Note the supra- and sub-oesophageal pleuro-visceral cords, the uncrossed external pallial nerves and the vestige of the velum retractor muscle. $\times 20$ approx.

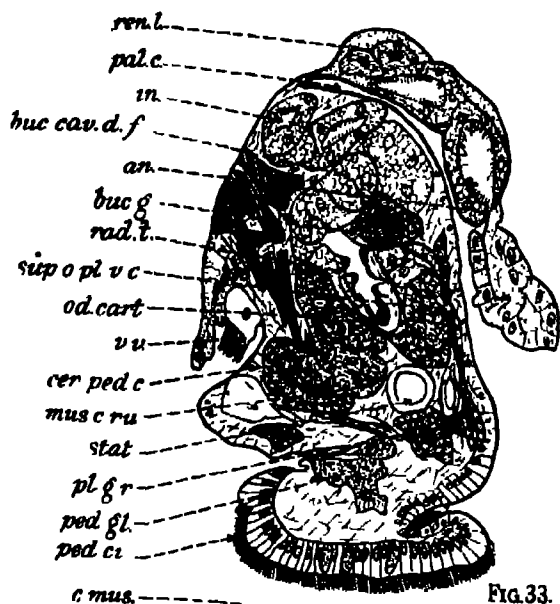


FIG. 33.

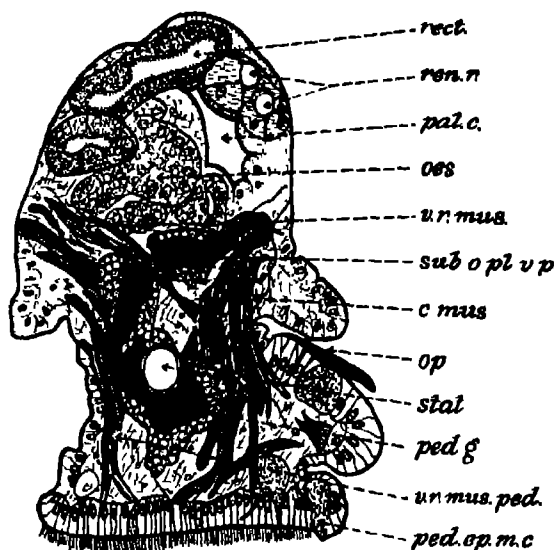


FIG. 34.



FIG. 37.

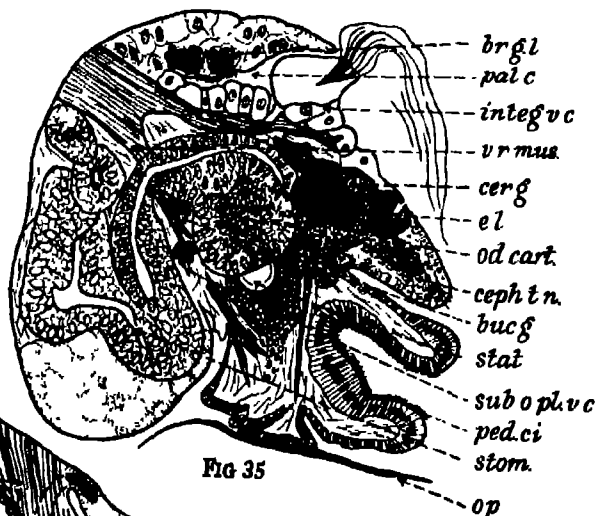


FIG. 35.

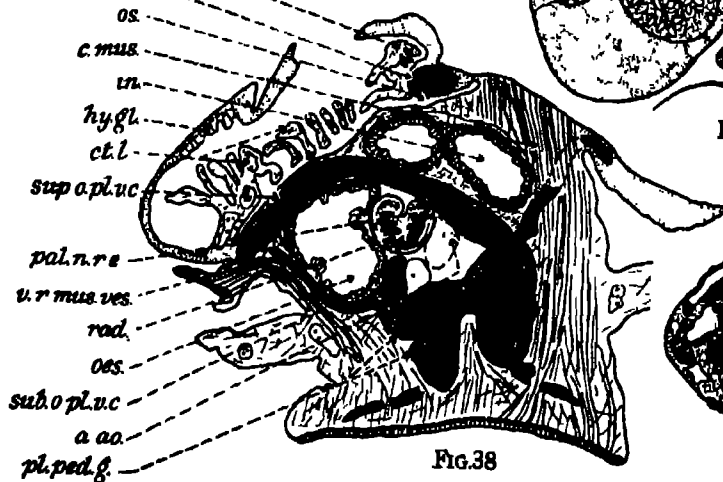


FIG. 38.

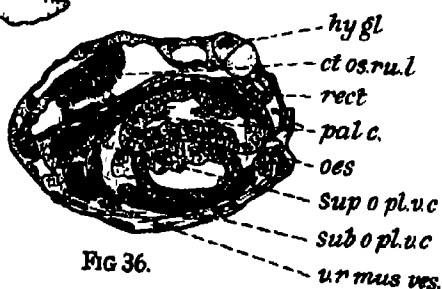


FIG. 36.

VI—THE DIGESTIVE SYSTEM OF *AMPHIOXUS* (*BRANCHIOSTOMA*) *LANCEOLATUS*

By E. J. W. BARRINGTON, M.A., B.Sc.

Department of Zoology, University College, Nottingham

(Communicated by E. S. Goodrich, F.R.S.—Received 9 March 1937)

[PLATE 28]

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1—INTRODUCTION

In view of the interest of *Amphioxus* as a primitive Chordate type, and its wide use in zoological teaching, it is remarkable that so little should be known of the structure and physiology of its digestive system. The early treatise of DELAGE and HÉROUARD (1898) gives little information concerning the mid-gut and the so-called "liver" beyond referring to their green colour, which is ascribed without further elucidation to the presence of secretory granules, while the hind-gut "ne présente rien de particulier". PIETSCHMANN (1929), in his recent excellent account of the Cephalochorda, can give little further information. The epithelium of the "liver" and "stomach" are described as composed of ciliated cells with granulated cytoplasm, but no suggestion of regional differentiation in the various parts of these organs is given. Of the function of the hinder region of the alimentary system nothing can be said beyond a reference to the spiral movement imparted to the food by the ilio-colon ring, while HAMMAR's

statement, based on an embryological study (1898), that the "liver" is homologous with the liver of the higher Chordata, is accepted without question. The essentially physiological monograph of FRANZ (1927*b*) is equally uninformative. It is, then, evident that this alimentary system demands a complete investigation both from the structural and functional points of view, and it has, in fact, been impossible to deal with all the problems which have suggested themselves. In its present form the work provides a description of the ciliary mechanisms of the post-pharyngeal regions of the gut, together with some account of the cytology of the epithelium and of the digestive enzymes secreted by it, and discusses in the light of this description the probable mode of operation of the mechanisms and the function of the various parts of the system; in conclusion, the homology of the "liver" is discussed, and a new interpretation of this organ suggested. It is hoped to undertake in the near future a comparative study of the cytology of the alimentary canal of the lower Chordata, and the cytological portion of the present work is therefore not to be regarded as exhaustive.

It is a pleasure to acknowledge my indebtedness to Professor E. S. GOODRICH, F.R.S., for the interest which he has taken in the progress of this work, and for enabling me to use the Oxford table at the Plymouth Laboratory of the Marine Biological Association, where the observations on the living material were carried out. I desire also to express my best thanks to Dr. E. J. ALLEN, F.R.S., and the staff of the Laboratory for the very complete facilities which I enjoyed there, and to Professor C. M. YONGE for reading and criticizing the first draft of this paper.

2—METHODS

For the study of fresh material three main methods have been used. First, animals have been studied under the dissecting microscope as they lay in a suspension of carmine in sea water. The progress of the carmine through the alimentary canal can be clearly seen, and this method alone, at least with very small and transparent animals, is very illuminating. Secondly, similar observations have been carried out upon animals which have been partially dissected. This involves decapitating the animal, trimming away the myomeres on each side up to the level of the roof of the body cavity, and, when necessary, displaying the interior of the gut by a lateral incision along its wall on one or the other side. Finally, pieces of excised tissue have been examined under the microscope in the usual way.

For cytological purposes entire animals or small pieces of the gut have been fixed in the "Susa" fixative of Heidenhain, Bouin, Carnoy (for glycogen), Flemming-without-acetic (for fat) and mercuric formol, and have been stained chiefly with iron haematoxylin, Mallory's triple stain, mucicarmine and iodine. For general purposes by far the best results were obtained with "Susa" followed by haematoxylin or Mallory's stain.

The other experimental details will be described under the appropriate headings.

3—PRELIMINARY OBSERVATIONS

The problems with which this work is concerned can best be introduced by a brief description of the general form of the alimentary canal and of the course of the food through it, so far as this can be observed in an entire living specimen resting in a weak suspension of carmine in sea water.

The pharynx (fig. 1, *ph.*) is continued backwards into a short and narrow oesophagus (*oes.*) which passes into a wide mid-gut (*ma.*); at the point of junction there arises the mid-gut diverticulum (*div.*) which extends forwards along the right side of the pharynx. The mid-gut passes into the short region termed by VAN WIJHE (1916) the ilio-colon ring (*icr.*), and from this the hind-gut (*hg.*) passes straight to the anus. According to VAN WIJHE it is uncertain whether the ring should be regarded as belonging to the hind-gut or to the mid-gut. In the light of the facts to be described below, the non-committal terms "mid-gut", "hind-gut" and "mid-gut diverticulum" seem preferable to such terms as "stomach", "intestine", "liver" and "hepatic diverticulum" which have been used by various writers, and the former will therefore be employed here throughout, as they have been by FRANZ (1927*a*).

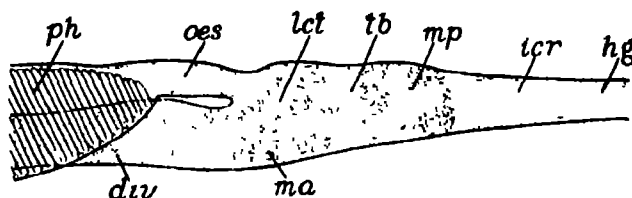


FIG. 1—Alimentary canal of *Amphioxus*, to show the chief regions.

MÜLLER (1844) has described the mid-gut and diverticulum as greenish in colour, but this depends a good deal upon the illumination. More commonly they present an opaque yellow-brown colour, although a green tinge is certainly sometimes visible. The oesophagus and the ilio-colon ring are clear (fig. 1), the greater portion of the hind-gut is dark, although less so than the mid-gut, while near the hind end of the hind-gut the wall again becomes clear (fig. 7, *cl.*). VAN WIJHE (1916) has shown that this appearance of the fresh tissues can to some extent be imitated in whole preparations by differential staining with carmine and aniline blue, the mid-gut and diverticulum then appearing greenish while the oesophagus and the hind-gut are rose. This he ascribes to differences in the structure of the cytoplasm in the several regions, but he does not describe these differences beyond pointing out that the numerous inclusions in the cells of the mid-gut are much larger than those of the hind-gut. The inference is, of course, that the greenish colour is due to the inclusions of the mid-gut and diverticulum being stained by the aniline blue.

The role of the ciliary mechanisms of the oral hood and pharynx has been fully described by ORTON (1913). He has shown that food particles enter the pharynx

in the current of water set up by the lashing of the lateral cilia of the gill bars, are collected by the frontal cilia on these bars, and then transported by them into the dorsal groove of the pharynx along which they are carried back into the oesophagus. A food collection of minor importance is effected by the wheel organ in the oral-hood cavity, some particles falling out of the main stream and becoming drawn against the ciliated tracts which compose this organ. These particles are transported in mucus to the peripharyngeal bands (which also receive particles from the anterior end of the endostyle) or are drawn into the pharynx in the main stream through the velar aperture. The larger particles are arrested on the oral-hood cirri which are kept folded over one another during the act of feeding, and thus is effected a selection of the finer particles for transmission into the oral-hood cavity.

The food cord formed in this way passes backwards through the oesophagus, and on arrival in the mid-gut usually drops sharply downwards towards the opening of the diverticulum (fig. 21, *fc.*). It does not enter this, however, but instead continues to pass backwards and finally arrives at the junction of the mid-gut and the ilio-colon ring. Here it may be arrested for a short time as a result of the "sphincter" which exists at this point (see p. 283), while fresh material continues to enter from the pharynx, but it soon passes on into the ring itself. The immediate result of entry into this latter region is that the cord begins to rotate by ciliary action on its longitudinal axis and becomes thrown as a result into spiral coils, the direction of this rotation being anti-clockwise to an observer facing the anterior end of the animal. Since the cord is continuous, this rotation is communicated to that portion of it lying in the mid-gut, the length of the cord affected by this rotation depending upon such accidental mechanical factors as the tension of the cord at the moment and the amount of material contained in it. The movement is a striking one and has been referred to by several writers (e.g. MÜLLER 1844; VAN WIJHE 1916; etc.), but it has not always been appreciated that the ilio-colon ring is the sole propulsive agent. Thus according to RICE (1880) the cilia of the "stomach" are so disposed that they force the food into a rope-like body and cause it to rotate, while ANDREWS (1893), describing the passage of the cord through the centre of the "stomach" in *Assymetron* (which may, of course, differ from *Amphioxus*), writes that it is now revolving rapidly from right to left and continues to do so throughout the next division of the digestive tract. To continue with the present account, the cord continues to pass backwards, and so long as the portion behind the ilio-colon ring remains continuous with the portion within the ring it continues to be affected by the rotation. Sooner or later, however, a portion breaks off, and it then no longer rotates, a fact which again illustrates that the ring is the propulsive agent. Once such a portion has broken off, it begins to pass slowly down the hind-gut, and its rate of progress can be measured by noting the time at which it passes the several myomeres. One such portion, selected at random, broke off from the main mass at 11.23 a.m., at which time it was opposite the third myoseptum behind the atriopore, and was expelled at 1.27½ p.m., having thus passed down the

hind-gut in 2 hr. 4½ min. Some ten myomeres had to be passed on the way, and most of the passage was slow and regular; towards the end, however, movement was suddenly speeded up, and the last three myomeres were passed in 3½ min. The existence of a muscle sphincter at the anus is well known (PIETSCHMANN 1929), but it is not this which is responsible for the terminal acceleration, for this can be observed to take place while the anus remains open and without any perceptible muscular movement at all. Nor, it may be added, does careful examination of the living animal suggest that peristalsis plays any part in the passage of the food mass down the rest of the hind-gut. It will be shown that the ciliation of the hind-gut is actually sufficient to explain not only the steady movement but also the sudden acceleration at the hind end.

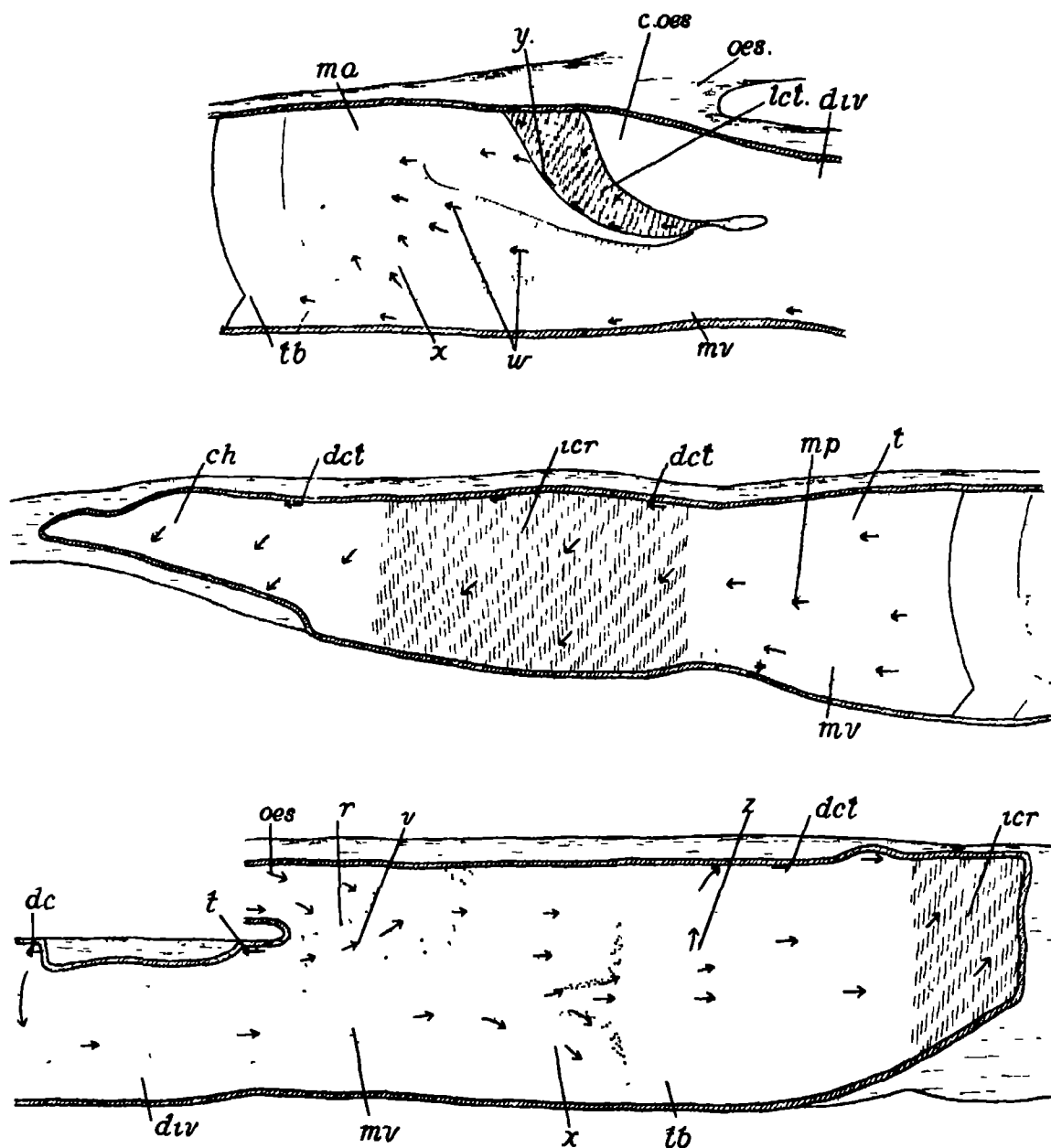
The comparatively rapid transit of the food mass down the hind-gut presents a certain difficulty, for WEISS (1890) and SCHNEIDER (1899) have referred to the absorption of carmine by the cells of this region, and it is difficult to see that the time of transit is long enough for such absorption to take place. Indeed, the times recorded here are probably above rather than below the normal average, for the animal was resting motionless. Movement, which must occur during natural conditions, certainly diminishes the time of transit, as may be seen when an animal is excited and swims vigorously for a few moments. Such activity usually results in the extrusion from the anus of some of the contents of the hind-gut, doubtless as a result of the compression of the gut by the contracting myomeres. According to PIETSCHMANN (1929) the passage of food from mouth to anus may take place in as short a period of time as 1 hr., a striking contrast with the Ascidian *Tethyum* in which, according to BERRILL (1929), the food takes about 35 hr. to pass from branchial sac to anus at 15° C.

It is clear that a number of questions are suggested by the above considerations: If the food cord does not enter the mid-gut diverticulum, what is the function of the latter, and what relation does it bear to the liver of the higher Chordata? Where are the digestive secretions produced, and how are they brought into contact with the food in the food cord? What is the significance of the rotation imparted to the latter by the ilio-colon ring? Where and how does digestion and absorption occur, and what is the function of the hind-gut? It is the object of the present work to provide some answer to these questions.

4—THE STRUCTURE AND CILIARY MECHANISMS OF THE ALIMENTARY CANAL

(i) *The Mid-gut Diverticulum*

The diverticulum is very compressed laterally, so that the roof and floor are very narrow in comparison with the depth of the lateral walls. An examination of the fresh organ shows that it contains a brownish, mucus-like material adhering closely



FIGS. 2-4—Views of the interior of the alimentary canal of *Amphioxus* to show the ciliary currents, combined from a number of dissected specimens. Fig. 2: interior of the anterior half of the mid-gut, with the right wall removed. Fig. 3: interior of the posterior half of the mid-gut, ilio-colon ring and anterior end of the hind-gut, with the right wall removed. Fig. 4: interior of the diverticulum, mid-gut and ilio-colon ring, with the left wall removed.

to the walls, and if a portion of the organ is cut open along one side and the whole laid flat upon a slide with the internal surface upwards (fig. 5), it can be seen that this material is kept in motion by the action of cilia. In such a preparation the coloration of the wall of the diverticulum is not uniform; the characteristic brown colour of the epithelium extends over two areas corresponding to the lateral walls (*lat.*), and these are separated by narrow and lighter bands extending along the roof (*md.*) and floor (*mv.*) of the diverticulum. It is in these lighter areas that the ciliary activity is strongest.

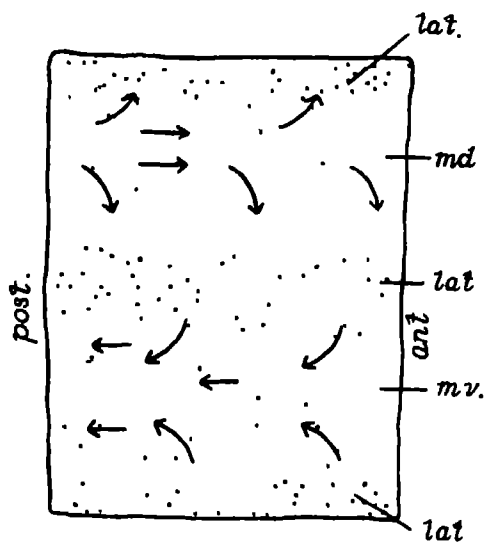


FIG. 5—Ciliary currents on the inner wall of the diverticulum.

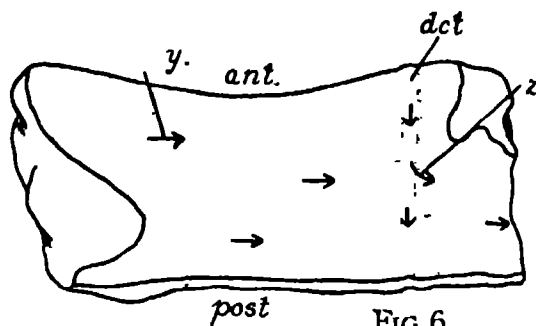


FIG 6

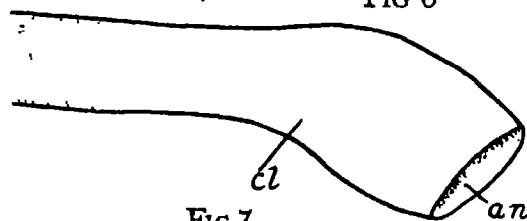


FIG 7

FIG. 6—Ciliary currents on the inner wall of the ilio-colon ring.

FIG. 7—Hind end of the hind-gut.

In flat preparations the currents are rather confused owing to the formation of eddies resulting from the depression of the narrow roof and floor below the level of the lateral walls. The situation is, however, that there is a strong ciliary current which beats along the floor of the diverticulum backwards towards the mid-gut (*mv.*), while another current, less strong, is directed forwards along the roof (*md.*). Particles in the vicinity of the ventral current are drawn into it, while particles travelling in the dorsal current tend to pass out from it on to the lateral walls, a difference due to the existence of a diffuse ciliary current directed downwards from the dorsal current to the ventral current (fig. 5). This downward current is so weak that there is a tendency, particularly noticeable after the addition of carmine, for particles to come to rest and accumulate along the lateral walls, but it must be remembered that these observations give a somewhat false impression of the conditions in the living animal, for there the lateral walls are vertical instead of horizontal as in these preparations. It may be assumed that under such conditions material will be much less likely to come to rest on the lateral walls, and that the general tendency inside the diverticulum will be

for all the contents to become drawn into the ventral current. This is confirmed by an examination of the diverticulum under approximately natural conditions, as is illustrated in fig. 4 which is of an animal in which the diverticulum and mid-gut have been exposed by removal of the myomeres and pharynx, and have then been opened along the left side. Carmine particles are swept into the diverticulum dorsally (*t.*) at its origin from the mid-gut (see p. 299), but a short distance forwards (at, for example, the point marked *dc.*) some of these can be seen to leave this dorsal current and to be swept straight downwards into the ventral current (*mv.*), and so backwards out of the diverticulum again.

The nature and source of the contents of the diverticulum may conveniently be studied in transverse sections of this organ stained in Mallory's triple stain. The organ itself is lined, like the rest of the gut, by a single layer of slender columnar cells resting on a thin layer of vascular connective tissue. BOZKE (1935) has described in the latter a layer of spindle-shaped smooth muscle fibres, running chiefly in a circular direction and associated with two autonomic nerve plexuses, this apparently applying only to the diverticulum, mid-gut and oesophagus. The lumen of the diverticulum is found to contain a variable quantity of a material which stains for the most part blue, although some portions of it display a reddish tint. That the blue-stained material is produced in the organ itself as an intracellular secretion appears certain, for the epithelium is largely composed of cells containing inclusions which are stained a similar colour. There are actually two distinct types of such cells, the first and more conspicuous occupying the greater portion of the epithelium of the diverticulum shown in fig. 41, Plate 28. This type is slender in form and possesses an elongated granular nucleus situated near the base of the cell and with a small prominent nucleolus. In haematoxylin preparations the body of the cell above the nucleus, with the exception of a narrow region at the free extremity, appears to be occupied by irregular vacuoles (*sa.*), but these are seen in Mallory preparations to be occupied by numerous small vesicles which are stained blue.

The second type of cell is quite clearly differentiated from the preceding. In shape it is more swollen, particularly in the region above the nucleus, while the cell inclusions are in the form of granules (fig. 39, Plate 28 *sg.*) which stain intensely with haematoxylin and blue with Mallory's stain, and extend from immediately above the nucleus to the free extremity of the cell. The most interesting characteristic of this type of cell, however, and one which will be discussed further below (p. 307) is the form of the nucleus (*ln.*). This is situated a little above the average level of the nuclei of the other cells; in shape it is much more spherical than they, and it possesses a single prominent nucleolus which is appreciably larger than the nucleolus of the first type.

For convenience these two types of cells will be referred to as type A (smaller nucleolus) and type B (larger nucleolus). They are further illustrated in several figures. In fig. 40, Plate 28 a group of type B cells is seen in the mid-gut, and the con-

spicuousness of the nucleoli (*ln.*) of the former in contrast with those of the surrounding cells is clearly visible. Fig. 34, Plate 28 is from a preparation of the diverticulum in which the nucleus (*ln.*) of a type B cell stands out in sharp contrast with the nuclei of the other type, while the swollen outline of the cell (*sb.*) is just distinguishable.

In view of the agreement between the staining reaction of the material in the lumen of the diverticulum and that of the inclusions of these cells, it may safely be inferred that the latter are secretory, and that the brownish material observed in fresh preparations takes its origin from them. It may be noted that the presence of these cell inclusions here and elsewhere, and their response to aniline blue, explains the observation of VAN WIJHE, mentioned above (p. 271), on the differential staining of whole mounts with carmine and aniline blue; it will be shown below, however, that blue-stained inclusions are also to be found, although less abundantly, in the epithelium of the hind-gut.

The two types of cells occur side by side in the epithelium, but generally their distribution is such that one or the other predominates. In the diverticulum itself, the type B cells are concentrated in the roof and floor, in the regions corresponding to the lighter bands seen in fresh preparations, with the result that the type A cells, although these extend all round the wall, are more conspicuous on the lateral walls. The distinction between these regions is further emphasized by the cells of the roof and floor being much shorter than those of the lateral walls. Something of this differentiation of the wall of the diverticulum has been noted by FRANZ (1925), according to whom the nuclei dorsally and ventrally, both here and in the mid-gut, are short and arranged in one layer, while laterally they are longer and arranged in several layers. He did not, however, notice the differentiation of the cells themselves, nor does he seem to have appreciated the existence of two distinct types of nuclei. In fact, he interprets the differences in appearance as due to the occurrence of less cell proliferation dorsally and ventrally than laterally, and regards this as a primitive character of simple guts. LANGERHANS (1876) has referred to the existence in the epithelium of the mid-gut and diverticulum of some cells filled with large granules, the remaining cells possessing a finely granular zone between the nucleus and the free border. He does not refer to the nuclei of these cells, but his figure shows that the former type of cell has a larger nucleus and a more prominent nucleolus than the latter type. It is probable that these cells represent the type B and type A cells respectively, but it is difficult to be certain of this from his description. PIETSCHMANN (1929) gives a figure (his fig. 72) after KRAUSE (to whose original work the present writer has not been able to obtain access) which shows two types of cells in the epithelium of the diverticulum, one with small dark granules and the other with lighter vesicles; these clearly represent the type B and A cells respectively, but the nuclei of the two types of cells are shown in the figure as being identical in appearance—faintly granular and with a prominent nucleolus. Finally, SCHNEIDER (1899) described vacuoles contained in the cells of the lateral wall of the diverticulum above the nucleus,

but believed these to be excretory, for he found that when carmine or iron solutions were injected into the tissues of the animal the substances appeared later in the vacuoles (see also p. 293). JORDAN (1904) showed, however, that such a reaction did not necessarily imply an excretory function, but was equally characteristic of secretory cells, the material in question passing into the cells from the blood in company with the material needed for the elaboration of the secretion. YONGE (1926*a, b*) has pointed out that it is possible to identify iron or other colouring matter in the secretory cells of the digestive glands of Crustacea, Insecta and Gastropoda after the substance has been injected into the tissues. SCHNEIDER's work, therefore, is not a proof of the occurrence of excretion in the diverticulum, and, in view of the existence of a well-developed excretory system in the form of nephridia, may be accepted as confirming the interpretation of the diverticulum cells as secretory. Moreover, the writer has found that a variety of digestive enzymes can be extracted from the organ (see p. 286), and their secretion can safely be regarded as composed of those enzymes.

It has been mentioned above that part of the contents of the diverticulum stains reddish, but there is no such clear correlation between this material and any cell inclusions as there is in the case of the blue-staining material. It is particularly conspicuous in the lumen of the hind-gut, of the oesophagus and of the hyperpharyngeal groove, and this suggests that it is merely material which has been swept in from outside, for it will be shown below that some material undoubtedly does occur free of the main food cord in the oesophagus and the mid-gut, and even in the diverticulum. However, presumed secretory granules which stain red are found in parts of the hind-gut epithelium (p. 284), and the possibility that this material is in part a secretion cannot be entirely dismissed. Finally, the epithelium of the diverticulum is seen in sections to be ciliated, the ciliation being stronger dorsally and ventrally than laterally; this is in agreement with the observed strength of the ciliary currents. As far as can be made out, the cells both here and elsewhere in the mid-gut and hind-gut do not bear more than one cilium each.

(ii) *The Mid-gut (anterior)*

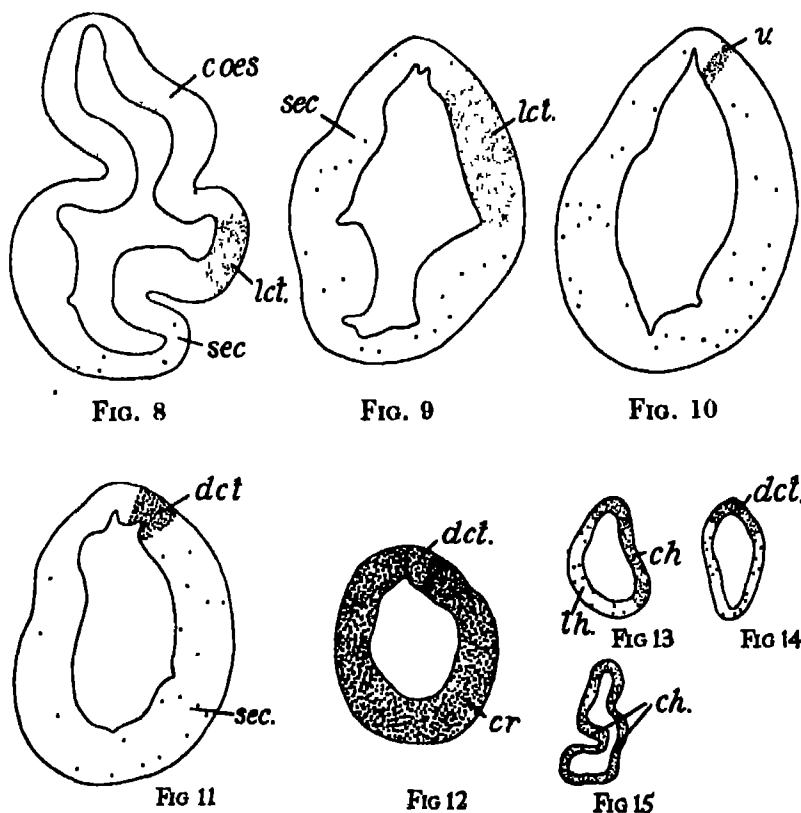
The mid-gut can be taken to extend from the junction between the oesophagus and the roof of the diverticulum to the beginning of the ilio-colon ring, and is divided into two approximately equal regions by a transverse light band (fig. 1, *tb.*) which runs transversely immediately posterior to the hind end of the floor of the diverticulum. On histological grounds it would probably be more accurate to speak only of the region behind this band as the mid-gut, since the region anterior to it, as will be shown, is a complex of tissue related in part to the oesophagus and in part to the diverticulum, but in practice the simpler morphological definition is more convenient. Owing to the elaboration of this region, it will be convenient to describe the anterior and posterior halves of the mid-gut separately, beginning with the former and describing the left side first.

The light band which has been seen to mark the floor of the diverticulum is continued backwards as far as the transverse band. As it approaches this it becomes depressed to form a conspicuous groove which is terminated at the latter by the upward sloping of the floor. A strong ciliary current passes backwards along this band (fig. 2, *mv.*) and groove, this current being, of course, a direct continuation of the ventral current already observed in the diverticulum. At the end of the groove it is directed upwards partly as a result of the slope of the floor. Carmine particles travelling in this current may be observed to undergo a sudden acceleration shortly before reaching the actual termination of this groove, at about the point *x*. in fig. 2. This acceleration is brought about by cilia on the walls of the groove, and particularly on the left wall, at which point there is an area of very prominent cilia grading off into the indistinct general ciliation of the groove of which it is evidently a special development. The effect of this ciliation is not only to accelerate the passage of material out of the groove, but also to direct it backwards and obliquely upwards, as shown by the arrows at the area *x*, in which it is to some extent aided by the slope of the floor. Not all the material driven along the groove is affected in that way, however, for particles travelling in the middle of the current away from the action of the lateral cilia pass straight backwards across the transverse band into the hinder division of the mid-gut, along the floor of which they continue to move backwards. The special ventral ciliated tract, however, ends at the anterior face of the transverse band, and from that point backwards movement is much slower, being due only to the general ciliation of the epithelium which has a backward trend in all parts of the mid-gut.

On the left wall of the anterior half of the mid-gut there is a clear area, roughly triangular in form, and visible even in undissected specimens (fig. 1, *lct.*). Examination of the inner surface in this region shows that this area marks the presence of a tract of exceptionally conspicuous cilia (fig. 2, *lct.*), which begins in front as a narrow band underlying the hind end of the oesophagus, and thus actually extending on to the left wall of the diverticulum, and widens behind until it extends upwards to the roof of the mid-gut. This tract is sharply defined by being depressed below the general level of the epithelium, as is well seen in horizontal section (fig. 37, *lct.*, Plate 28), and this demarcation, combined with the clearness of this area and the prominence of its cilia, makes this tract one of the most striking features of the gut. However, it seems to have been overlooked by all previous writers with the exception of VAN WIJHE (1919) who has briefly described in the metamorphosing *Amphioxus* an area of similar shape and position, appearing at the same time as the "liver" rudiment. According to him the area contains both ciliated and secretory cells, and he has regarded it as the pancreas. Unfortunately he gives no illustrations of this region, but it will be seen below that at least in the adult the cells of this tract are quite distinct from the typical secretory cells of the gut, although a clearly marked tract of secretory cells runs along its dorsal edge. There seems, therefore, no justification for regarding it as pancreatic, and it will be referred to here as the "lateral ciliated tract". The direction

of the beat of the cilia composing it is such as to drive particles downwards and backwards (fig. 2), and carmine grains in this region tend to collect in a cord which moves steadily backwards (*y.*) along the lower edge of the tract.

Carmine particles also move backwards along another path which extends underneath the lateral ciliated tract and is separated from it by a darker area. This tract is actually not very well defined except in so far as the area composing it is lighter than the surrounding epithelium, but its existence is well shown by the movement of the particles. These follow the direction of the arrows (fig. 2, *w.*), passing backwards and curving slightly upwards. Below this tract a dark area extends down to the lighter region of the floor of the mid-gut. Finally, the epithelium lying above and anterior to the lateral ciliated tract, between it and the beginning of the diverticulum, is light (fig. 2, *c.oes.*), while behind the tract the epithelium becomes darker.



FIGS. 8-15—Selected transverse sections, all from the same specimen, to show the distribution of the ciliated cells with dark nuclei. For further explanation, see text.

Reference to transverse sections shows a considerable degree of histological differentiation in this region. The clear ventral band along the floor resembles the corresponding band along the floor of the diverticulum in containing many secretory cells of type B. On either side of this band and extending a short distance up the lateral walls are secretory cells of the other type, giving the dark appearance to this region as they do

in the diverticulum. Thus, the lower portion of this region of the mid-gut (fig. 8, *sec.*) is secretory, and exactly resembles the latter in its structure, and is in reality a continuation of it. Cilia are well developed along the floor, but are scanty towards the upper limit of the type A cells. The lateral ciliated tract is easily recognized in sections by its dense ciliation and the distinctive character of the cells composing it. These cells lack the inclusions found in the secretory cells, while their nuclei are much more densely granular and stain, therefore, very darkly, a small nucleolus being just recognizable. These cells are illustrated in fig. 41, Plate 28 which is from a transverse section at a level close to the point of junction of the oesophagus and the diverticulum. At this level the cells are confined to a narrow band (*lct.*), the orientation of which is shown in fig. 8, *lct.*; this section is at a level just posterior to the junction of the oesophagus and diverticulum. Farther back this band broadens and extends dorsally (fig. 9, *lct.*), this corresponding to the observed shape of the organ (fig. 2). In the hinder region of the anterior half of the mid-gut, behind the lateral ciliated tract, the secretory cells, predominantly of type A, gradually extend upwards and this accounts for the dark appearance of the wall. The ciliated cells with dark nuclei have practically disappeared, but it is just possible to trace a few backwards to the left of the mid-dorsal line (fig. 10, *v.*). The subsequent fate of this narrow tract of cells will be considered below. It should be understood that ciliary activity is not confined to the restricted area where the cells with dark nuclei are located. It appears rather that these nuclei are correlated with an exclusively ciliary activity on the part of the cell, the lighter nuclei being associated with secretion and absorption. It has been mentioned that the area of the left wall anterior to the lateral ciliated tract is light in appearance; it is really a continuation of the epithelium of the oesophagus, and, like the latter, is composed of cells devoid of blue-staining inclusions and possessing slender, darkly-staining nuclei. These nuclei resemble those of the cells of the lateral ciliated tract in their dense granulation and in the very small size of the nucleoli, but appear to stain a little less darkly than do the latter. Irregular inclusions are sometimes seen in these cells, but it has not been possible to determine their nature.

The right wall of the anterior half of the mid-gut (fig. 4) calls for little comment. There is no specialized ciliation corresponding to the lateral ciliated tract, but there can be detected on the wall a gentle backward and upward current (*v.*) leading away from the right wall of the diverticulum, the current finally ending by diffusing indefinitely over the wall. The path of this current, like that of the current *w* on the opposite wall (fig. 2), is not defined structurally apart from the fact that the wall here is somewhat lighter than elsewhere, nor is it composed of the cells with dark nuclei. Immediately anterior to it a dark band is prominent (fig. 4, *r.*), and this may be regarded as marking the boundary on this side between the mid-gut and the oesophagus. Farther backwards the secretory cells soon extend upwards (fig. 9, *sec.*), and towards the hind end of the anterior half of the mid-gut (fig. 10) the disposition

of the cells becomes more or less symmetrical, nearly the whole of the wall being secretory. It is clear, however, that most of the anterior half is extremely asymmetrical in its minute structure.

(iii) *The Mid-gut (posterior)*

The light band which separates the two halves of the mid-gut is difficult to identify in transverse sections, but in horizontal sections it is easily recognized as a zone of cells with only slight traces of secretion separating two areas of close-packed secretory cells. Across this band, as has been seen, the particles on the wall of the gut are driven backwards. Behind the band there is a general backward movement all over the walls of the gut (fig. 3, *mp.*), the current along the floor (*mv.*) being a little stronger than that along the walls. One new feature, however, concerns the roof of this region. Particles crossing the band at or above the level indicated by the letter *z* in fig. 4 are seen to turn abruptly upwards with a considerable acceleration, and to be drawn out of sight along the roof of the gut. The abrupt upward movement is not due to any disposition of the cilia on the wall, but is due to the particles coming under the influence of a strong backward current (*dct.*) which sets in along the roof at about this point. Its presence is shown also by the particles which are flung out of it farther backwards. This current is best seen in specimens which have been opened from the left side; unfortunately it cannot be observed in pieces of the mid-gut which have been removed from the animal as the roof always tears in this region as a result of its firm attachment and delicate structure.

Examination of sections, however, confirms the existence of a specialized ciliary tract at this point, for slightly to the left of the mid-dorsal line there may be seen (fig. 11; fig. 35, *dct.*, Plate 28) a narrow but distinct area of the epithelium composed of ciliated cells of the type with dark nuclei, a groove in the epithelium being also usually visible. These cells can be traced forwards to the narrow tract of cells which has been described above (p. 281) as passing backwards from the upper end of the lateral ciliated tract (fig. 10, *v.*). At about the level of the transverse band these cells become more numerous and conspicuous and form into a groove (fig. 11, *dct.*), and it is here that, in fresh preparations, the strong backward current first comes into evidence. This special tract will be referred to here as the "dorsal ciliated tract", although its actual position (fig. 11) is here slightly to the left of the mid-dorsal line. Presumably the narrow tract of cells which connects it with the lateral ciliated tract also produces a backward current, but as the effect of this was not noted in dissected specimens it is evidently only weak.

The remainder of the wall of this half of the mid-gut is in function essentially secretory (fig. 11, *sec.*), although it is also ciliated throughout. Immediately behind the transverse band, secretory cells of type A predominate, but farther back the type B cells become conspicuous, at first ventrally. Fig. 40, Plate 28, is taken from a trans-

verse section through the level at which these cells first become visible in the mid-ventral line (*ln.*) with the type A cells (*sa.*) extending upwards on either side. Finally the type B cells come to predominate all round the wall apart from a narrow band high up on the right wall where type A cells remain conspicuous. On the left wall in the middle portion of its length the secretory cells are less abundant than elsewhere and the epithelium here is largely composed of cells compact in structure and lacking in any obvious characteristics. This area corresponds to an area (fig. 3, *t.*) on the left wall which in dissected specimens commonly exhibits a distinctive yellow-green tinge. It is impossible to say what its special function, if any, may be.

(iv) *The Ilio-colon Ring*

The mid-gut leads into the ilio-colon ring, a region which has attracted the attention of several previous workers (MÜLLER 1844; ANDREWS 1893; GOLDSCHMIDT 1905; VAN WIJHE 1916). It has been explained above that this region is responsible for imparting a rotation to the food cord, and the cilia effecting this movement are very conspicuous when the ring is slit open in situ (figs. 3 and 4, *icr.*). The direction of the beat of the cilia is oblique, the beat passing obliquely downwards and backwards on the left side, and obliquely forwards and upwards on the right side. This is shown not only by the actual direction of the beat, which is clearly visible, but by the course followed by carmine particles. These pass gently backwards along the wall of the hinder half of the mid-gut, but on entering the ring the rapid oblique movement is assumed. These cilia do not complete the ciliary mechanisms of the ring, for the dorsal ciliated tract also extends backwards from the mid-gut (*dct.*). Its presence can be detected both by the direct backward movement of particles in the dorsal region, as in the mid-gut, and also by the direct observation of excised tissue. It is possible to remove the ring, open it by a longitudinal incision, and spread it flat; in such a preparation (fig. 6) the tract is visible as a clearly marked narrow depression (*dct.*). Particles dropped on the preparation are driven upwards (*y.*) by the main cilia of the wall, but some enter this groove and immediately change the direction of their movement and pass along it, soon, however, to pass out again on the other side (*z.*) and continue their vertical movement. In such preparations the obliquity of the main ciliary beat over the wall is not very distinct, owing to the distortion which these pieces of tissue undergo on being cut.

The point of junction of the mid-gut (fig. 29, *mp.*) and the ring (*icr.*) is marked by a thickening of the epithelium (*th.*¹). This results in the existence here of a circular ridge, shown in horizontal section in fig. 38, *th.*¹, Plate 28, which by projecting into the lumen acts as a partial sphincter and causes the temporary arrest of the food cord. On some occasions it has appeared as though the actual rate at which the food passes on into the hind-gut were controlled by the relaxation of this sphincter, although it is difficult to decide how far this is due to the mere pressure of the food mass. There

is no muscle sphincter here corresponding to that found at the anus, but the smooth muscle fibres and autonomic nerve plexuses described by BOEKE (p. 276) would probably suffice to account for such movements of the gut wall. In sections the epithelium of the ring has a very characteristic appearance, being composed of slender and closely crowded cells (fig. 12; fig. 33, *cr.*, Plate 28) with a particularly strong and dense ciliation. They are of the same general type as the ciliated cells with dark nuclei already described, but the elongated and rod-like nuclei stain conspicuously darker than do the former, a small nucleolus being only just distinguishable against the background of granulation. At one point on the circumference, slightly to the left of the mid-dorsal line, the nuclei are less crowded and stain less intensely, and are confined nearer to the base of the epithelium (fig. 12; fig. 33, *dct.*, Plate 28). At this point also the ciliation is less dense, while aniline blue and mucicarmine reveal the existence of a few mucus cells. This region is clearly the dorsal ciliated tract which is thus structurally as well as functionally distinct from the rest of the epithelium of the ring.

(v) *The Hind-gut*

The cells composing the epithelium of the hind-gut are at the anterior end intermediate in height between those of the ring and those of the mid-gut. Running down the length of the roof of the hind-gut is a tract of the ciliated cells with dark nuclei which is a continuation of the dorsal ciliated tract and maintains the same histological characteristics as that (fig. 14, *dct.*). At the anterior end of the hind-gut the ciliated cells with dark nuclei are not confined to this dorsal tract, but extend down on the left side towards the mid-ventral line (fig. 13, *ch.*). The remainder of the wall, i.e. the greater portion of the right side, is composed of cells (*lh.*) with nuclei which are less densely granular and in which the nucleolus is more prominent, while the cilia are shorter and less abundant. Within these cells there are many inclusions.

Closer examination of the less strongly ciliated region shows it to be composed of two types of cells. Firstly, there are cells in which the nuclei are slender (fig. 36, *sn.*, Plate 28), but less so than those of the strongly ciliated region, and possess a small but prominent nucleolus. Above the nucleus there are distributed in the cytoplasm a number of vesicles which, unstained by haematoxylin, are stained blue by Mallory's stain; these vesicles resemble in their staining reactions the vesicles found in the type A cells of the mid-gut and diverticulum, although they are much less conspicuous. The second type of cell possesses a larger, more rounded and somewhat granular nucleus with a conspicuous and larger nucleolus (*ln.*), the nucleus being situated at the base of the cell at the same level as the other type. This second type of cell, which seems to be most abundant at the anterior end of the hind-gut, is further characterized by the presence in the cytoplasm of small granular inclusions (*sg.*) which stain darkly with haematoxylin and reddish with Mallory. It seems certain that this type, and pro-

bably also the preceding type, are secretory, and in a general way they clearly resemble respectively the type B and type A cells, making allowances for differences in size. The granular inclusions differ in one respect, however, for they stain blue with Mallory in the mid-gut and diverticulum, while another difference is that large and irregularly shaped inclusions (*ab.*) are found in this area of the hind-gut epithelium. These inclusions are probably connected with the absorption of food (p. 293).

It follows from the above that at the anterior end of the hind-gut (fig. 13) there are to be distinguished two areas of the epithelium distinct in both function and structure. The one (*ch.*) is characterized by its strong ciliation and by the relative absence of inclusions, while the other (*lh.*) is less strongly ciliated and contains many inclusions related to secretion and absorption. Passing backwards, the relative proportions of these two areas gradually change, the boundary between them being displaced upwards along the left wall until the strongly ciliated cells are confined to the dorsal tract (fig. 14, *dct.*). In other words, these latter cells occupy a triangular area of which the base lies at the anterior end of the hind-gut while farther back the apex passes into the dorsal tract, at a level roughly one-third of the length of the hind-gut behind its anterior end. Behind this point and for the greater part of the rest of the length of the intestine, the proportions remain as in fig. 14. At the hind end, however, the cells with dark nuclei again extend downwards and finally occupy the whole circumference (fig. 15, *ch.*). The epithelium here is very shallow, and the closely crowded nuclei occupy about two-thirds of its total depth.

By direct observation (fig. 3) it can be seen that the strongly ciliated cells on the lateral wall at the anterior end of the hind-gut drive particles obliquely backward (*ch.*) as do the cells of the ilio-colon ring, the result being to drive them on to the area occupied by the less strongly ciliated cells. It may safely be inferred that the dorsal tract continues the backward movement already seen in the mid-gut and ring, and that the downward extension of the strongly ciliated cells at the hind end, which corresponds, incidentally, with the terminal clear area of the epithelium (fig. 7, *cl.*), is responsible for the sudden acceleration (p. 273) which drives the food cord residue out of the anus (*an.*). The dorsal tract is no doubt active in driving the mass down the hind-gut, but its further probable function will be discussed below. Definite confirmation of these inferences is difficult as the hind-gut is very thin and easily tears on removal; nothing, however, has been observed which conflicts with them. The hind-gut naturally tears along the mid-dorsal line where it is attached to the body wall, and there is commonly seen along the torn edge a strong backward current which clearly represents the current of the dorsal tract. The impression gained from such pieces of tissue is that on the other parts of the wall—i.e. the less strongly ciliated area—the effect of ciliation upon movement is only slight.

5—THE DIGESTIVE ENZYMES

(i) *Methods*

The interpretation of the ciliary mechanisms to be advanced below implies the secretion in the diverticulum and other parts of the alimentary canal of digestive enzymes, and the following results are designed to provide evidence for this. At the beginning of the investigation it was hoped that it would be possible to compare the secretory activity of the different regions of the gut, but so far as the present results are concerned this has not been possible, for the analysis of the method by which the food and secretions are transported through the gut has shown that material in some stage of digestion might be expected to occur in all parts of the mid-gut and hind-gut, so that the mere fact of a given region exhibiting digestive activity need not imply that the enzyme concerned was actually produced there. Theoretically, this last difficulty could be overcome by thoroughly cleaning the tissues before grinding them, but in practice the gut wall is too delicate to admit of this. The considerable activity of the diverticulum extracts provides, however, good evidence for the production of all types of digestive enzymes in this region, for it has been shown above that relatively little material passes into it from the remainder of the alimentary canal. The cytological evidence, it will be recalled, suggests the production of secretions in all three regions, and shows also that the boundary between the diverticulum and the mid-gut is less pronounced cytologically than it appears to be morphologically.

For these experiments the diverticulum, mid-gut, hind-gut and portions of the pharynx were dissected out, the food cord removed, the various tissues ground up in a little distilled water with silver sand, and the mixture left to stand overnight with a drop of toluol added; the resulting extracts were made up with water in the proportion of 0.1 g. of tissue to 5 c.c. of water (2% extract). In addition to the identification of the enzymes, a few experiments were set up to determine the variation of activity of the protease, lipase and amylase over a stated pH range, and further experiments along these lines are in progress. Clark and Lubs's buffers were used for all experiments except those concerned with the lipase, for which the B.D.H. Universal buffer was employed. The pH was measured by means of the B.D.H. Capillator before and after incubation, and the actual pH was taken to be the average of the two readings.

(ii) *pH of the Alimentary Canal*

The pH of the gut contents was estimated by means of the capillator, using bromothymol-blue for indicator. Freshly caught specimens were dissected out of water, and portions of the gut contents mixed with a drop of distilled water, a drop of the indicator being added to an equal drop of the resulting mixture. Material from the ilio-colon ring and the hinder end of the mid-gut gave a value of pH 6.7, and material from the hind-gut values of 7–7.1. The pH of the diverticulum was tested in a similar

way except that, owing to the absence of solid masses of food from this region, portions of the entire organ were removed and squeezed up with a drop of water; the pH was found to be 6.2. As a check on this method, a piece of the mid-gut was removed and squeezed with water; the pH was found to be 6.7. No explanation can at present be suggested for the marked acidity of the diverticulum. SCHNEIDER (1899) seems to have observed the same condition, for he reports that after feeding animals with blue litmus powder the "liver" and the part of the gut nearest to the opening of that organ were red, the rest of the gut being blue. This is in agreement with the above results.

It follows that digestion proceeds at a pH ranging from 6.7-7.1, for it will be argued below that digestion and absorption must be mainly confined to the mid-gut and hind-gut; indeed, if the diverticulum were an important site of digestion, its pH would be expected to agree more closely with that of the mid-gut and hind-gut. It may be added that the pH of the lumen of the gut in the Ascidian *Tethyum* (BERRILL 1929) ranges from 6.8 to 7.4.

(iii) *The Digestion of Carbohydrates*

For all experiments upon the carbohydrate enzymes, tubes were made up to contain 0.25 c.c. of extract, 0.5 c.c. of buffer solution, 1 c.c. of substrate and one drop of toluol, and the mixtures incubated for about 48 hr. at 35° C. At the end of this time the degree of digestion was estimated either by adding 0.5 c.c. of the digest to 1 c.c. of Benedict's quantitative solution and completing the titration with standard glucose solution, or by titrating 0.5 c.c. of the digest with sodium thiosulphate solution according to the method of HAGEDORN and JENSEN as modified by BOYLAND (1928). The activity of the respective digests is expressed below as the difference between the titration readings for the boiled control and the active mixtures, in terms of c.c. of the glucose or thiosulphate solutions per 0.5 c.c. of digest mixture.

The results of one of several preliminary experiments are shown in Table I, and indicate the presence of an amylase in extracts of the diverticulum, mid-gut, and hind-gut but not of the pharynx.

TABLE I—AMYLOCLASTIC ACTIVITY EXPRESSED AS C.C. OF STANDARD GLUCOSE SOLUTION. SUBSTRATE: 1% STARCH

	pH	Active	Control	Difference
Diverticulum	7	0.90	2.80	1.90
Mid-gut	7	1.45	2.75	1.30
Hind-gut	7	0.65	2.80	2.15
Pharynx	7	2.75	2.75	0

The difference in the degree of activity of the three active tissues is not necessarily significant, for the total weight of tissue is always small and the concentration of the extracts, nominally 2%, can therefore only be approximate. These differences are, however, being further investigated.

The amylolytic activity of the diverticulum is expressed graphically in fig. 16 (method of HAGEDORN and JENSEN), which indicates an optimum activity between pH 6 and 6.5.

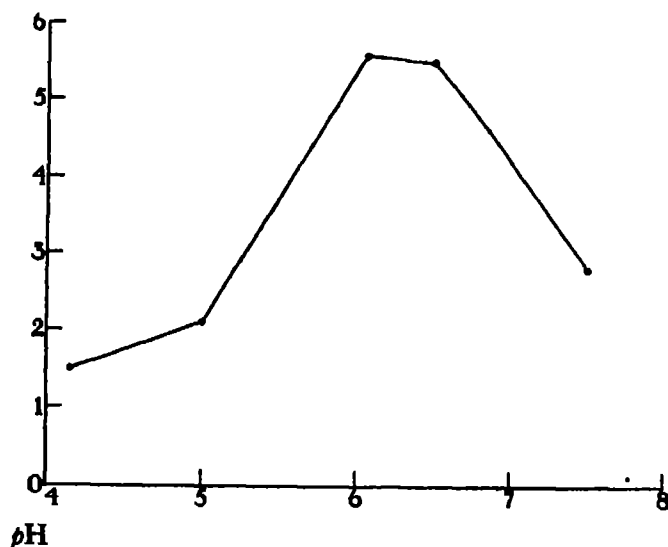


FIG. 16—Amylolytic activity, by thiosulphate titration, of the diverticulum. Substrate: starch. Ordinates: c.c. of thiosulphate solution.

The capacity of the animal for digesting certain other carbohydrates is shown in Table II, the various substrates being tested by the above two methods except in the case of maltose and lactose, for which Barfoed's solution was used, following the procedure of ROAF (1908). The sawdust and gum arabic mixtures were incubated for 6-7 days.

TABLE II—DIGESTION OF CARBOHYDRATES, EXPRESSED AS C.C. OF GLUCOSE OR THIOSULPHATE SOLUTIONS

	c.c. of glucose solution				c.c. of thiosulphate solution			
	pH	Active	Control	Difference	pH	Active	Control	Difference
1% glycogen	6	1.55	2.70	1.15	7	1.56	3.95	2.39
2% sucrose	6	2.35	2.80	0.45	7	2.23	4.12	1.89
1% salicin	6	2.55	2.75	0.20	7	2.54	3.78	1.24
1% inulin	6	2.80	2.80	0	7	3.77	3.80	0.03
0.05 g. sawdust	6	2.65	2.65	0	7	4.78	4.83	0.05
5% gum arabic	6	2.65	2.65	0	7	5.24	5.22	-0.02
2% maltose	6	Reduction	No reduction		7	Reduction	No reduction	
2% lactose	6	Reduction	No reduction		7	Reduction	No reduction	

These results provide evidence for the digestion of glycogen, sucrose, salicin, maltose and lactose, in addition to starch, but not for the digestion of inulin, sawdust or the pentosan gum arabic. These may be compared with the results obtained by previous workers for the Tunicata. For *Ciona*, YONGE (1925) obtains positive results for starch,

glycogen, sucrose, salicin and amygdalin, and negative for inulin, raffinose, cellulose, maltose and lactose, while for *Tethyum* BERRILL (1929) obtains positive results for starch, sucrose, maltose and lactose, and negative for cellulose. The latter author finds the optimum for the amylase to lie between pH 7 and 7.5, appreciably higher than in *Amphioxus*; apart from this, conditions in the two groups are essentially similar.

(iv) *The Digestion of Fats*

For the identification of a lipase, tubes were made up to contain 0.3 c.c. of active or boiled extract, 0.3 c.c. of 0.1% triacetin, 1.5 c.c. of buffer solution, and one drop of toluol. The lipoclastic activity was determined, after incubation for 48 hr., by direct titration of the fatty acids with N/100 caustic soda using phenol-phthalein as indicator, each active mixture being titrated against its corresponding control. Positive results were obtained for the diverticulum, mid-gut and hind-gut, and negative for the pharynx, the results of one experiment being shown in Table III.

TABLE III—LIPOCLASTIC ACTIVITY, EXPRESSED AS C.C. OF N/100 CAUSTIC SODA.
SUBSTRATE: 0.1 % TRIACETIN

	pH	Active	Control	Difference
Diverticulum	7.9	0.33	0.16	0.17
Mid-gut	7.9	0.41	0.27	0.14
Hind-gut	8.0	0.28	0.22	0.06
Pharynx	8.0	0.22	0.22	0

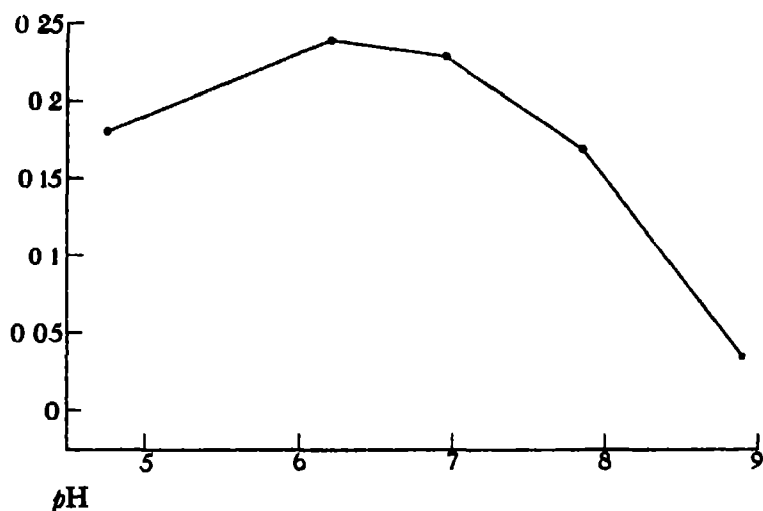


FIG. 17—Lipoclastic activity of the diverticulum. Substrate: 0.1 % triacetin.
Ordinates: c.c. of N/100 caustic soda.

Experiments to determine the optimum pH of the lipase, using diverticulum extracts, failed to give very precise results, apparently owing to the weakness of the enzyme. They provided, however, good confirmation of the existence of a lipase in these extracts, and the results of one of the experiments are therefore shown in fig. 17. The optimum would appear to lie close to the pH range at which digestion takes place.

A lipase has been identified in *Ciona* (YONGE 1925) and *Tethyum* (BERRILL 1929), but these authors did not investigate the pH optimum.

(v) *The Digestion of Proteins*

The proteolytic digestion proved the most troublesome to investigate in the preliminary experiments, but satisfactory positive results were eventually obtained by means of a method involving the liquefaction of gelatine, the results of one representative experiment being shown in Table IV. Each tube in this experiment contained 0.1 g. of gelatine powder, 1.5 c.c. of buffer solution, 0.3 c.c. of extract and one drop of toluol; these mixtures were incubated for 48 hr., and the Table states their condition after they had then been placed on ice for 35 min.

TABLE IV—PROTEOLYTIC ACTIVITY, AS INDICATED BY THE LIQUEFACTION OF GELATINE

	pH	After 35 min. on ice		pH	After 35 min. on ice
Diverticulum	2.0	Solid	Diverticulum	8.3	Fluid
Diverticulum	2.7	Solid	Diverticulum	9.1	Fluid
Diverticulum	4.3	Solid	Hind-gut	8.5	Fluid
Diverticulum	5.0	Solid	Pharynx	8.7	Solid
Diverticulum	5.7	Semi-fluid	Control	5.5	Solid
Diverticulum	7.2	Fluid	Control	8.7	Solid

The results indicate the existence in the extracts of the diverticulum and hind-gut, but not of the pharynx, of a protease which may for the present be described as of the "tryptic" type. In other experiments, extracts of the mid-gut gave comparable positive results.

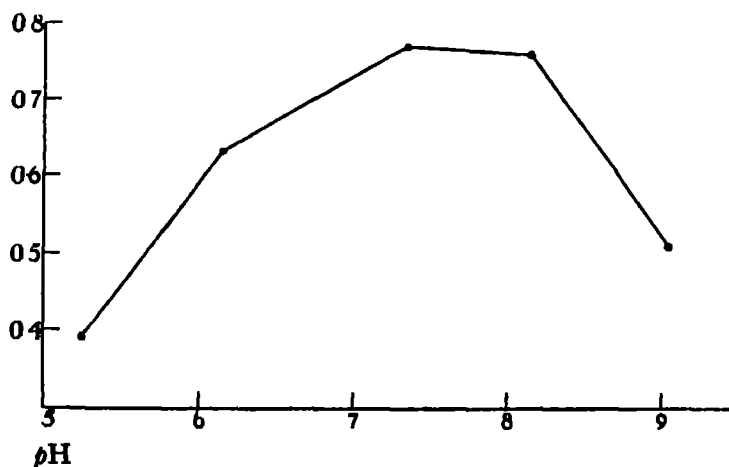


FIG. 18—Proteolytic activity of the diverticulum. Substrate: casein.
Ordinates: c.c. of N/100 caustic soda.

The proteolytic activity of the diverticulum was further examined by means of the formol-caustic soda titration method, following in essentials the procedure described

elsewhere (BARRINGTON 1936). These experiments confirmed the presence of a protease of the "tryptic" type but, as with the lipase, failed to give precise readings for the optimum point. The results of one of these experiments are shown in fig. 18; the optimum probably lies in the region of pH 8.

YONGE (1925) has described in *Ciona* a protease active in neutral and alkaline media, while in *Tethyum* (BERRILL 1929) the protease is active from pH 6 to above 10, with an optimum probably near to 8 or 8.5. The conditions are, therefore, essentially similar in the Tunicata and *Amphioxus*, and it may be concluded from the above that, so far as present information goes, there is no fundamental difference between the enzymes of the two groups.

6—ABSORPTION

For the study of absorption, animals were fed upon carmine, iron saccharate (according to the method described by YONGE 1926*a*), precipitated gold, indian ink, and fat, the last involving placing the animals for periods of 15 min. at a time in a weak emulsion of olive oil in sea water, and subsequently fixing in Flemming-without-acetic.

In a specimen which had remained in a carmine suspension for 48 hr., numerous minute red particles were easily seen in unstained sections of the hind-gut, situated in the distal region of the cells of the weakly ciliated region. Another specimen which had remained for 24 hr. in carmine showed some red particles in the cells of the hind-gut; many other colourless inclusions were also seen in this region (see below). No evidence of the presence of carmine was seen in any other part of the alimentary canal of these two specimens. In a third, which had remained for four days in carmine suspension, the weakly ciliated region of the hind-gut was coloured a diffuse red throughout most of its length, while towards the anterior end of this region many red particles could be seen in the cells; the dorsal ciliated tract was clearly differentiated by its lack of colour. Very fine dark granules were visible on the lateral walls of the diverticulum, and also in the cells of the mid-gut, chiefly on the right wall in the latter. In the light of other evidence to be presented below, these may be regarded as carmine, their very small size concealing their red colour. In view of the independent evidence for the taking up of solid material, the red particles in the cells of the hind-gut may be regarded as carmine particles, although, theoretically, they might be due to the staining of intracellular inclusions by carmine in solution (HORSTADIUS 1933). It may be added that the absorption of carmine "in its very finest granules" was observed long ago by WEISS (1890).

In a specimen which had been for four days in a suspension of indian ink, small masses of ink were visible in the cells of the hind-gut; very fine particles of ink could be seen in the epithelium of the mid-gut, mostly on the right wall, and of the diverticulum, where they were particularly concentrated along the middle of the lateral walls. Many of the particles in the latter organ were conspicuously grouped in small

spheres, corresponding to the secretory droplets of the cells (p. 276). A second specimen, treated for two days, showed a considerable amount of ink similarly arranged in the diverticulum; ink was also present in the epithelium of the mid-gut, on the right side and ventrally, but only very occasional particles could be seen in the hind-gut. A third specimen, treated for two days, showed a rather small amount of ink in the cells of the hind-gut; particles were also distinguishable in the diverticulum and the posterior half of the mid-gut, but were far less conspicuous than in either of the two previous animals. On the whole, indian ink seems not to be taken up very readily.

In two animals which had remained for two days in an iron saccharate suspension, the Prussian blue reaction was given by the cells of the hind-gut, only a slight reaction being given by the cells of the dorsal ciliated tract. In one of the specimens large masses of the iron compound could be seen in the cells, and a thin layer of the material was closely applied to the surface of the epithelium, the whole giving a very convincing picture of absorption from such a layer as is referred to below. In this specimen some diffuse coloration was also to be seen in parts of the mid-gut, but not in the diverticulum or oesophagus. Another specimen, treated for two days, showed small masses of the compound in the epithelium of the hind-gut; in the cells lying along the middle of the lateral walls of the diverticulum, a blue colour showed very clearly in the region of the secretory droplets.

In a specimen which had remained in a suspension of precipitated gold for 4 days, large masses of gold were easily seen in the cells of the anterior region of the hind-gut. Similar large masses were seen in the mid-gut, many small particles and occasional large masses in the diverticulum, and occasional particles even in the oesophagus. In a second specimen, similarly treated, masses were seen in the anterior region of the hind-gut; the most remarkable feature of this specimen, however, was the presence of numerous large masses in the epithelium of the mid-gut and the diverticulum, together with numerous small particles, the gold being very much more abundant here than in the hind-gut. In both specimens gold was easily seen in other parts of the body, notably in the subepithelial layer and blood vessels of the gut, and in the gonads, and it is clear that this material is taken up very readily indeed.

In two animals which had been fed upon fat, droplets of the fat were to be seen either scattered through the body of the epithelial cells of the gut, or concentrated at their bases. In the hind-gut they were distributed through the body of the cells, with no clear distinction between the strongly and weakly ciliated areas, while there was some concentration at the base. In the diverticulum, scattered droplets were found in the distal regions of the cells, although less conspicuously than in the hind-gut, while there was considerable concentration at the bases of the cells. Fat was scarce in the mid-gut, except on the left upper wall, and there was no evidence of concentration here. Some fat also occurred in the oesophagus. In a specimen which had been fixed in Flemming without preliminary feeding, the distribution of fat droplets was similar but more clearly defined; fat was scattered through the body of cells in the hind-gut,

but was very little concentrated at the base, while in the diverticulum there was considerable concentration at the bases of the cells, ending abruptly in a very striking way at the point where the diverticulum passes into the mid-gut, and an almost complete absence of fat from the body of these cells. Fat was very scarce in the mid-gut, except dorsally in the posterior region. If it be assumed that the scattered and concentrated conditions indicate respectively absorption and storage, all three specimens imply considerable absorption in the hind-gut with some storage, and considerable storage in the diverticulum, with the possibility of some absorption in the latter and in the mid-gut. The distinction is not, of course, a perfectly sharp one.

The above specimens have been described separately in order to indicate the range of variation which occurs amongst them. They show quite clearly that the epithelium can ingest solid material, and that this ingestion occurs at least in the hind-gut and especially in the weakly ciliated region. This is in agreement with the argument to be advanced in the next section, that a layer of food and secretion is distributed over the epithelium in this region by ciliary action. In animals which are fixed in the ordinary way without preliminary artificial feeding, large and irregular inclusions are consistently seen in the distal region of the cells of the weakly ciliated area; they are easily distinguished (fig. 36, *ab.*, Plate 28) by their size and shape from the secretory granules (*sg.*) which are also present in this epithelium, as has been seen above, and in some instances a clearer, vacuole-like area can be seen around them. These inclusions, which can be seen even in unstained sections, are usually confined to the distal half of the cell body, while the secretory granules extend down towards the base. These particles, occasionally to be seen also in the dorsal ciliated tract, can thus be interpreted as masses of the mixed food and secretion, the digestion of which is completed within the cells. Such an occurrence of intracellular digestion would provide an exception to the general rule (YONGE 1937) that extracellular digestion has completely replaced intracellular in, amongst other groups, the Chordata, although it will be clear from other sections of the present work that the extracellular method must be important in *Amphioxus*, for the food becomes thoroughly mixed with extracellular secretions. Digestion is presumably merely completed inside the cell after preliminary action in the lumen of the gut, and the ingested extracellular secretion may well be sufficient for this purpose.

The appearance of the food materials in the cells of the mid-gut and diverticulum in most of the specimens presents a more difficult problem. So far as the latter organ is concerned, it seems certain that this cannot be entirely due to absorption. SCHNEIDER (1899), it will be recalled, showed that the "liver" cells came to contain carmine and iron after solutions had been injected into the tissues and not allowed to enter the lumen at all, while WEISS (1890) showed that carmine particles absorbed by the hind-gut were taken up into the blood vessels and subsequently deposited in the atrial epithelium and the nephridia. In some of the specimens described above, the absorbed material was easily identified in the blood vessels and gonads, and the peculiar

grouping of the ink particles into spheres in the cells of the diverticulum has been mentioned; staining with Mallory showed that these groups were contained within the blue-stained secretory droplets, and could scarcely have been absorbed from the lumen. Finally, minute granules of gold, carmine and ink could be traced from the cells into the small masses of secretion in the lumen, and since many of the cells in the neighbourhood were clearly spent secretory cells, there is a strong presumption that such granules were being removed in the secretion. The analysis of the ciliary mechanisms will show that they can only allow of the entry of relatively small quantities of food material into the lumen of the diverticulum; very little indeed is seen in sections in comparison with the mid-gut and hind-gut, and it is difficult to believe that the quantities are sufficient to provide for the absorption of as much material as is seen in the cells of the diverticulum. Taking all the above arguments into consideration, it may be concluded that most of this material has been transported in the blood stream. At the same time, it is impossible to exclude the possibility of some absorption occurring in the diverticulum, especially under conditions when considerable quantities of material are being swept over the walls of the gut (p. 298), and this would account for the occurrence in the diverticulum of occasional inclusions like those characteristic of the hind-gut. The problem must be considered in the light of the ciliary mechanisms, and will be returned to later (p. 299).

The situation in the mid-gut is obscure. A good deal of food material will be moving over the walls, and some absorption might well occur, especially in the posterior half, for here many particles are broken off from the rotating food mass when this is large enough to extend forwards out of the ilio-colon ring, but here also the possibility of transport of material in the blood cannot be neglected.

It may be concluded for the present that absorption occurs mainly in the hind-gut and especially in the weakly ciliated area, to a lesser extent in the mid-gut, and possibly, but to a still lesser extent, in the diverticulum. No special differentiation between secretory and absorptive cells has been observed, and it seems likely that all parts of the epithelium are capable of absorption, the limiting factor being the ciliary mechanisms. It is clear that the precise relationship between absorption and transport can only be decided by comparing a number of specimens which have been subjected to carefully controlled periods of starvation and feeding; it is hoped to continue the investigation along those lines.

7—THE OPERATION OF THE CILIARY MECHANISMS

(i) *The Mid-gut and the Mid-gut Diverticulum*

It now becomes possible to attempt the construction of a complete picture of the functioning of the post-pharyngeal region of the alimentary canal. The food cord has been seen to pass from the oesophagus into the mid-gut and to pass backwards through the latter partly, no doubt, under the influence of the mid-gut cilia and partly through

the pressure of fresh material being driven in from the oesophagus. It may or may not be arrested for a short time at the "sphincter", but it soon passes into the ilio-colon ring and is at once set into rotation.

To this rotating cord there have to be conveyed the digestive secretions which are produced all over the wall of the diverticulum. The cilia in this region tend to concentrate the material on to the floor, for although there is a forward current along the roof it has been seen that particles travelling in this tend to pass out from it and to be carried ventrally by the ciliation of the lateral walls. Along the floor a strong current conveys the material backward to the transverse band in the mid-gut. The diverticulum itself is too opaque to allow of direct observation of the movement of the secretions in the intact organ, but just anterior to the transverse band the wall of the gut is more transparent, and in exceptionally favourable circumstances, using animals from which the myomeres have been trimmed away, it is possible to watch the masses of secretion sweeping into view at this point. As they approach the band they can be seen to move

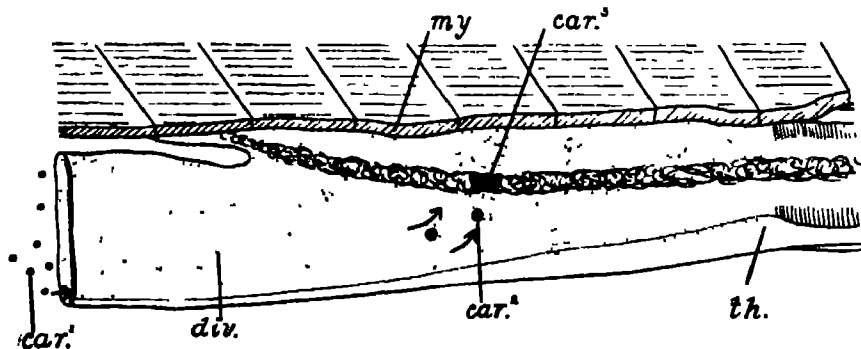


FIG. 19—Left side-view of *Amphioxus* to illustrate the method by which the secretions of the diverticulum are transferred to the food cord.

upwards a little towards the food-cord, this upward movement being clearly identical with a similar oblique upward and backward movement noted in the dissected specimens (fig. 2, x.). This brings the material so close to the rotating cord that it touches and adheres to the latter as it passes over it, and so becomes added to the cord of mucus and food.

The process can be demonstrated at any time in a convincing way by using an animal from which the anterior end has been removed to a level about half way or more down the diverticulum. The specimen is placed on its side in sea water (fig. 19) and a little carmine suspension added to the water with a pipette immediately in front of the cut end of the diverticulum (*car.¹*). Some of the carmine particles are swept into the diverticulum and along the floor by means of the strong ciliary current, and under the microscope their appearance anterior to the transverse band (*car.²*), their upward movement and their adhesion to the sticky food cord (*car.³*) are easily seen as has been described above for the secretion. In such an animal the effect of cutting is to slow down the ciliary activity, in addition, of course, to interfering with

the intake of material by the pharynx and oesophagus. While, therefore, the food cord continues to rotate slowly it will usually not pass backwards, and so gradually a number of carmine granules accumulate in the cord just above the transverse band (*car.*³).

The rotation of the food cord, however, also has other effects. In one exceptionally small and therefore very transparent specimen, resting naturally in a carmine suspension, the following points were observed: As the red food cord rotated, carmine particles would be seen to leave the main mass behind the transverse band to pass forwards along a roughly semicircular course on the left side in the dorsal region of the mid-gut, the direction changing towards the end of their course into a downward movement. The semicircular course had the appearance of free movement in space, as though the particles had been thrown off forwards from the left of the rotating cord, while the downward movement was of a quite different steady nature and suggested the action of a ciliary tract on the left wall. After they had been carried downwards a little way to about the level of the food cord, the direction of movement was again changed, this time backwards, and they joined up with the food cord. It is apparent that the downward and backward movement coincided exactly with the movement that would be set up by the lateral ciliated tract (the movements were actually observed before the interior of the mid-gut had been investigated, and led to the conclusion that some sort of ciliary organ must be present on the left wall), and it follows that part of the function of the latter is to collect and restore to the cord material which has been swept off from it. That material should break away from the cord is easy to understand, for the rotation is not a regular one, but involves much friction with the walls which would naturally tend to disrupt the surface of the cord. Further evidence that particles were breaking off from the surface was provided in this same specimen by the fact that as rotation continued the wall of the posterior half of the mid-gut acquired a red lining which at intervals was sloughed off and restored to the cord, only to be rapidly replaced, while conclusive evidence in support of this will be adduced below in connexion with the discussion of the function of the ilio-colon ring.

What is not so easy to understand is why the particles should pass forwards for a little distance after leaving the cord. Careful examination reveals no ciliary mechanism on the wall which would account for this, and the only other possible explanation seems to be that they are involved in a reverse eddy set up by the backward current in the ventral groove. There is justification for assuming the existence of such an eddy, for with the food cord blocking the passage between the mid-gut and the ilio-colon ring, the mid-gut is practically a closed chamber, and in view of the strength of the ventral current the existence of some forward eddy would seem inevitable. This problem was tested experimentally in the following way: The anterior and posterior ends of the animal were cut off, the myomeres trimmed away, and the mid-gut slit open along the right side, taking care to leave the food cord intact. The preparation (fig. 20) was then placed in a glass cell in a suspension of carmine in sea water and

covered with a cover-slip in such a way that the mid-gut was restored to its original condition of a closed space, with the difference that part of the right wall had in effect been replaced by the cover-slip. Particles of carmine were swept along (*mv.*) the diverticulum into the mid-gut and, on approaching the transverse band, were swept upwards and forwards (fig. 20, *s.*) towards the diverticulum, as though under the influence of a reverse eddy. The preparation was then removed and the food cord pulled out through the ilio-colon ring, the preparation then being replaced as before. The difference now was that the mid-gut was no longer a completely closed space, for there was now a wide posterior opening through the ilio-colon ring. The effect of this upon the movement of the carmine particles was at once apparent, for the forward eddy was practically non-existent; previously some particles had even re-entered the diverticulum (*t.*), but now there was only a slight eddy in the region of the transverse band.

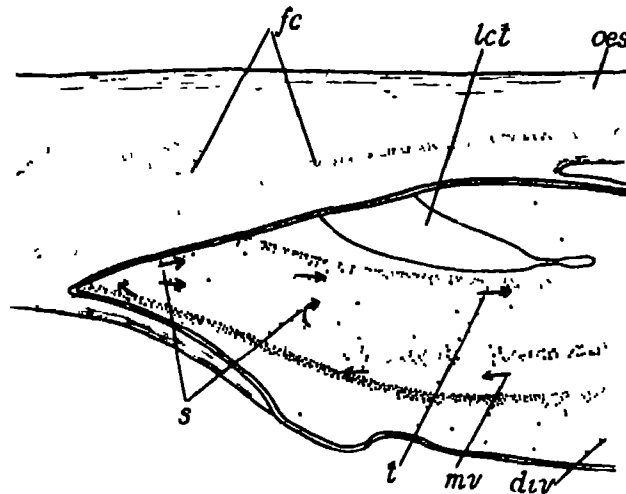


FIG. 20—Right side-view of the interior of the mid-gut to illustrate the production of reverse eddies.

It is unfortunate that the emission of particles from the cord and their return to it have only been clearly seen in one specimen, for none other sufficiently small has been secured. A slightly larger specimen was, however, still sufficiently transparent to show that during the rotation the wall of the mid-gut became densely red, particularly in the region of the lateral ciliated tract, while it was just possible to distinguish the blurred forward movement of occasional larger particles across the transverse band. In any case, there is no reason at all for regarding the movements seen in the very small specimen as in any way abnormal; indeed, according to the analysis suggested above, they are the inevitable result of the conditions obtaining in the mid-gut. It is difficult to see that the forward and return movement of the particles is of advantage to the animal, although it might be said that their passage through the gut is to this extent retarded, and thus more time allowed for digestion, but it is more

likely that the process is a by-product of the rotation of the cord. The primary objects of the latter appear to be firstly to mix thoroughly the food and the enzymes, and secondly to break off small particles from the mass for transmission down the hind-gut in order to provide for absorption. The second point, to be dealt with further below, rests upon the assumption that many of the particles broken off from the mass as it rotates in the mid-gut will not be carried forwards, but will be caught in the strong backward current of the dorsal ciliated tract by which they will be swept into the ilio-colon ring and so on into the hind-gut.

Before following their fate in the latter region, however, attention must be directed to another aspect of the function of the lateral ciliated tract; this concerns its extension forward to the hinder end of the diverticulum. If an animal, not too large, is examined while in a carmine suspension (fig. 21), it will often be seen that carmine is collecting

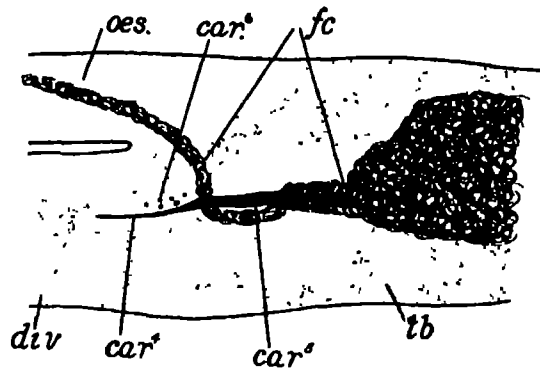


FIG. 21—Left side-view of the mid-gut of an entire *Amphioxus* to illustrate the collection of carmine by the lateral ciliated tract.

very conspicuously in an area ($car.^4$) which corresponds exactly to this anterior extension, and some particles ($car.^6$) can be seen to settle down in this region. From here they are no doubt continuously moving backwards to join the main cord ($fc.$), as has been explained above, but although the junction is visible ($car.^5$), the actual movement cannot be seen through the thickness of the body wall. To some extent this concentration of carmine would be made up of particles moving forwards in the mid-gut and which have not been trapped by the posterior portion of the organ, but it seems certain that this could not account for such a conspicuous and dense concentration. It must be remembered that the wall of the oesophagus is ciliated and it is inevitable that in a dense carmine suspension some material will be swept into the oesophagus and along its walls without being collected into the main cord. This has, in fact, been observed directly, by opening in the usual way an animal which had been for some time in a carmine suspension; particles were streaming down the wall of the oesophagus, and many of these were collecting in the anterior extension of the lateral ciliated tract. It thus appears that this organ helps to collect and convey to the main food cord material which passes down the oesophagus without being compacted into the cord.

A dense carmine suspension provides, of course, an excessively unnatural source of material. How far food material will enter the oesophagus outside the food cord under natural conditions it is impossible to say, but it seems inevitable that some will do so, and that much which does will tend to collect in the lateral ciliated tract. RICE (1880) describes the food of *Amphioxus* as consisting of any organic bodies available; these "sail along down the canal", commence to rotate as they approach the end of the cord, often "making uncertain efforts to escape", until gradually pressed into the mass. RICE appears to underestimate the effect of the collection of much of the food into a cord before it leaves the pharynx, and it seems possible that the "uncertain efforts to escape" may have been the movements of material broken off from the surface of the rotating cord. It may be added that in the same animal resting in a carmine suspension under apparently constant conditions, the anterior extension of the organ will be at one time full of carmine and at another time empty. This could be explained on the above lines by ascribing it to differences in the amount of carmine being taken in through the pharynx and oesophagus.

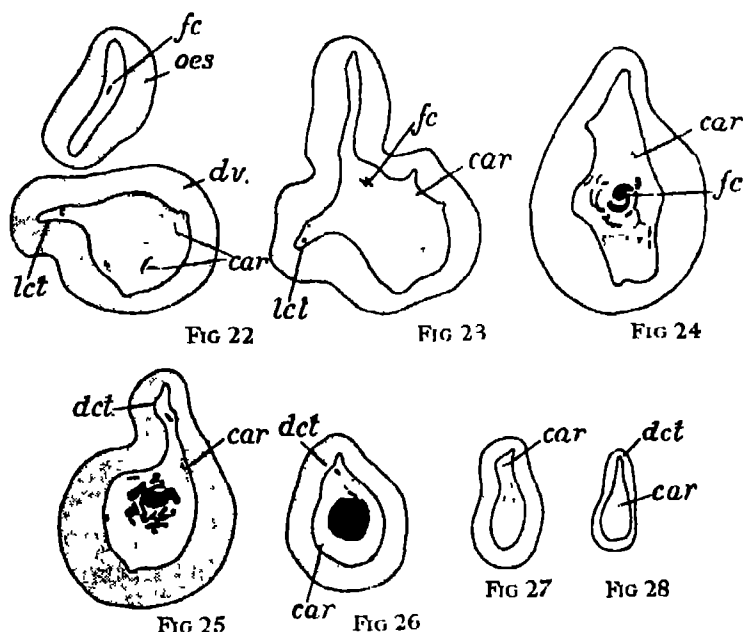
The action of the lateral ciliated tract is confined to the left side of the gut, and particles entering down the right side of the oesophagus will be more remote from its influence. It has been seen that there is a backward ciliary current on the right side (fig. 4, *v.*) which will naturally help to drive them backward, but the complication here is the existence of a strong forward current along the roof of the diverticulum (*dc.*). Owing to the fact that the diverticulum passes to the right of the oesophagus and pharynx, particles on the right side of the oesophagus will be much more influenced by it than will those on the left side. It is a fact that in transverse sections of animals which have been placed in a carmine suspension for some hours, carmine particles can be seen in the lumen of the diverticulum, and it must be the forward current along the roof of the diverticulum which is mainly responsible for this. The existence of this forward current is perhaps the most puzzling feature of the alimentary canal. It would be natural to suppose that its function was to convey material into the diverticulum in order that digestion and absorption could take place there, but against this must be set the existence of the lateral ciliated tract, which collects much material which would otherwise pass forwards into the diverticulum, the complete absence from the mid-gut of any forward current leading to the diverticulum, the elaborate mechanism for driving secretions out of the diverticulum and mixing them with the food cord, and the strong backward currents of the dorsal ciliated tract, ilio-colon ring and right wall of the hind-gut (see below). The point is of some interest, as it determines whether the organ is primarily secretory, like the "liver" of the Tunicata, or primarily both secretory and absorptive, like the digestive gland of certain invertebrates. The evidence is not quite conclusive, but taking into consideration the ciliary mechanisms as a whole, and the discussion concerning absorption (p. 294), the former interpretation seems likely to be the correct one; any material which enters the diverticulum, either from the wall of the oesophagus or in the reverse eddies, may possibly undergo absorption

if it is not removed again in the ventral current, but this would constitute a useful by-product of the digestive processes rather than a primary feature of it. It may be suggested that the primary function of the forward current is to promote a circulation of material in it and so assist in the collection of the secretions in the backward ventral current. Apart from this one factor, however, it will be appreciated that the ciliation in this region will tend to drive all material backwards towards the hinder half of the mid-gut where it will become added to the rotating cord. The adhesion of this material to the cord is probably more direct in practice than it might seem to be from the above account. All the contents of the gut are very sticky, and the least contact will cause adhesion. It may be supposed that the actual condition in the living animal comprises not so much a central main cord and a steady stream of material along the rest of the wall, as a main cord which collects all the other material through the medium of many irregular threads from the other parts of the wall, this collection representing, in fact, another function of the rotation. This can be confirmed by observation: If an animal is opened in the usual way, five or six threads will probably be seen extending from various parts of the lateral ciliated tract to the central cord, and if they are removed with a needle, they will rapidly reform.

It has been seen that the wall of the mid-gut is predominantly secretory. There is no difficulty in visualizing the addition of these secretions to the food mass, either after passing backwards from the more anterior regions, or, in the case of the secretions produced in the hinder half of the mid-gut, by direct adhesion. (The sloughing off of the lining of the epithelium has been described above in a very small specimen, and in this way the secretions will be carried on to the cord.) Animals are sometimes seen in which the main bulk of the food cord is concentrated in the ilio-colon ring and only a very slender cord extends forwards into the mid-gut; under such conditions it would seem that much of the secretion must pass backwards over the wall of the mid-gut, and not mingle with the food until it arrives in the ilio-colon ring. This, however, is merely a delay in the process of mixture described above, and no doubt there actually occurs every gradation between these two possibilities.

The main points in the above discussion can conveniently be recapitulated by reference to a series of transverse sections through an animal which was fixed while actively taking in carmine. Fig. 22 is of a section just anterior to the junction of the oesophagus (*oes.*) and diverticulum (*div.*). It shows a little massed carmine in the former (the main food cord, *fc.*), and many dispersed particles (*car.*) in the lumen of the diverticulum, predominantly on the right side, while a small concentrated mass of carmine is located in the anterior extremity of the lateral ciliated tract (*lct.*). It will be noticed that in the diverticulum there are many particles lying dorsally and, therefore, close to the dorsal forward current which, according to the above assumptions, will have driven them into the diverticulum. Particles are also accumulated ventrally, where the ventral backward current will tend to collect them and drive them out of the diverticulum into the mid-gut. Fig. 23 is of a section at a level

close behind the junction of diverticulum and oesophagus. The main food cord (*fc.*) has now dipped down to the level of the diverticulum; the particles (*car.*) on the right side of the lumen would have arrived there directly from the oesophagus. A small mass of carmine is again seen in the lateral ciliated tract (*lct.*). Fig. 24 is of a section at a level just anterior to the transverse band. A mass of carmine (*fc.*) lies in the centre of the lumen, and to this the separate cord of carmine formed by the lateral ciliated tract has now become joined. The anti-clockwise rotation of the mass is clearly shown; many particles are seen to be lying free of the main mass, and one group is sweeping dorsally (*car.*). It is easy to appreciate from this section that the rotating mass constitutes a focal point from which some particles are broken off and to which others are restored.



FIGS. 22-28—Selected transverse sections, all from the same specimen, to illustrate the distribution of carmine in the lumen of the alimentary canal of an animal fixed while actively taking in carmine.

Fig. 25 is of a section through the posterior half of the mid-gut. The central mass is seen, together with a few particles lying dispersed in the lumen, and it is also apparent that the particles which are moving dorsally are collecting in a small mass at the level of the dorsal ciliated tract (*dct.*) under the influence of which they may be expected to move back. This upward movement is no doubt the combined result of the rotation of the central mass and of the attractive force of the dorsal ciliated tract to which attention has already been drawn in the dissected specimens (fig. 4, z.).

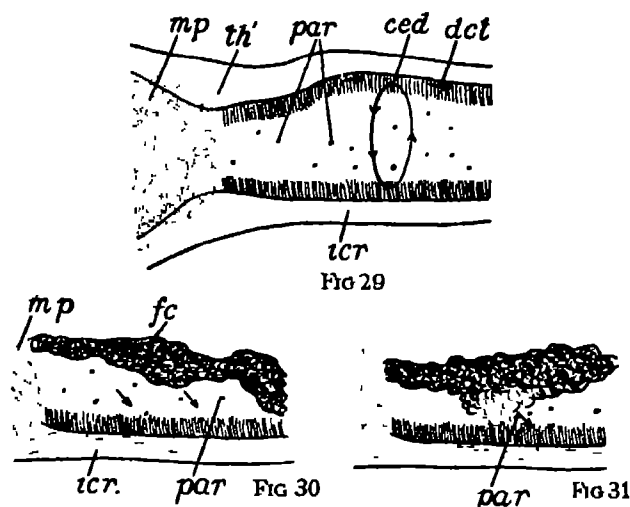
(ii) *The Ilio-colon Ring and the Hind-gut*

So far attention has been concentrated upon the mechanism by which the food and the secretions are brought together in the mid-gut, and there must now be considered the problem of how absorption is enabled to take place in the hind-gut. The solution of this problem appears again to lie in the rotation imparted to the cord by the ring. Let it be supposed that, as the mass rotates in the ring, particles are broken off as they are in the mid-gut. Then it is to be expected that these, together with those which have already been swept in from the mid-gut, would be swept out of the ring by the oblique ciliation of the walls and by the dorsal ciliated tract, the two sets of ciliary mechanisms acting in conjunction with each other. Particles travelling over the wall under the influence of the oblique ciliation will eventually be swept into the dorsal ciliated tract, and will then either leave the hind-gut in this or, after passing a little distance backwards, will be drawn out of it again on to the lateral wall, to repeat the process. It will finally be a matter of chance which of the two mechanisms is actually controlling their movement at the point when they leave the ring to pass into the hind-gut. Other particles will no doubt be moving in addition in the lumen, but these also will be irresistibly urged out of the ring, the point being that the ciliation in this region is such as to drive any particles out into the hind-gut.

Within the latter there are three ciliated areas which will influence the movement of these particles in three different ways. The triangular area of strongly ciliated cells on the right wall will drive particles, as a result of its obliquely backward beat, on to the weakly ciliated area where their rate of movement will be greatly reduced. Those particles travelling in the dorsal ciliated tract may be expected to pass farther back down the hind-gut; theoretically, they might be driven right out of the anus, but in practice it seems likely that many will be caught up on the lateral walls, for it must be remembered that these particles are very adhesive, while further secretions are being added to them from many of the cells. Thus the final result would seem to be the production in the hind-gut of a layer of material adhering to the walls and localized especially over the area where the ciliation, and therefore movement, is weakest. To this layer would be added other particles moving freely in the lumen. It is, indeed, impossible to estimate what proportion of particles would travel over the walls and what proportion would travel in the lumen, but all will tend to collect over that region of the epithelium where there is least ciliary activity, and it is that region that has been seen to provide the best evidence for absorption. The triangular shape of the ciliated area on the left wall of the hind-gut, and its oblique ciliary beat, are excellently adapted for driving particles over a maximum area of the hind-gut epithelium, and for preventing any tendency of the food particles to accumulate in one restricted area of the hind-gut.

For the above hypothesis some direct observational evidence is available. Fig. 29 shows an ilio-colon ring (*icr.*) removed intact and examined on a slide with the food

mass removed. Particles (*par.*) are seen to be moving in circular eddies (*ced.*) in the lumen (not obliquely, because they are here moving freely in the lumen and not along the walls), and at intervals they come under the influence of a strong backward current dorsally—the current of the dorsal tract (*dct.*)—and by that are driven a short way backwards. It is, then, clear that such particles would be driven out of the ring; it remains to show from where they have originated.



FIGS. 29-31—The ilio-colon ring in operation.

Figs. 30 and 31 show two stages in the passage of the food cord from the mid-gut (*mp.*) into the ring (*icr.*). In fig. 30 it is just entering (*fc.*); particles (*par.*) are in movement over the wall under the influence of the oblique ciliation. In this specimen the influence of the dorsal ciliated tract cannot be detected as the mid-dorsal line is obscured by the cut edges of the myomeres, the ring being viewed in situ. Fig. 31 shows the same a few seconds later. The food mass is nearly stationary, and at one point is being worn away by ciliary action into particles (*par.*) which are swept obliquely away, and thus, eventually, out of the ring. The fate of this material in the intestine has not been observed directly, but an examination of serial sections is relevant, and reference may again be made to the series of sections (figs. 22-28) showing the distribution of carmine in the alimentary canal. Fig. 26 is of a section through the ilio-colon ring. Particles are lying in the lumen and also in contact with the cilia on the walls (*car.*) as postulated above (although many are too small to be shown in the figure), while there is a distinct tendency for some to become concentrated in the neighbourhood of the dorsal ciliated tract (*dct.*). A section through the anterior region of the hind-gut (fig. 27) also shows particles (*car.*) present dorsally, and it is clear that these must form part of a stream of particles which is being driven backwards by the dorsal tract. Other particles are seen in the lumen of the hind-gut and also against the long cilia on the strongly ciliated side. Farther back absorption is occurring; particles (*car.*) are seen in the lumen and occasionally

lying against the wall and also occasionally in the region of the dorsal ciliated tract (*dct.*). Generally speaking, the effect of fixation seems to be to cause material lying against the wall to shrink away into the lumen, and probably some of the material seen in the lumen in these sections would be close to the epithelial cells in the living animal; this is, in fact, seen in other sets of sections. However, after allowing for such changes in position induced by fixation, the sections illustrated provide a good corroboration of the general account of the movement of food and secretions postulated above. Some of the minor details must necessarily be a little speculative; the essential factors in the present argument are, however, a separation of fine particles from the rotating cord and their transmission backwards by means of the dorsal tract and the ciliation of the walls over the absorptive areas, and for this there seems to be confirmation both in the evidence provided by fresh tissue and that provided by serial sections. No doubt some material would remain in the mid-gut, and this would account for the apparent absorption noted there.

One further point remains to be noted. The above account of the distribution of material in the hind-gut has not dealt with the situation which arises when from time

to time a mass of material breaks off from the rotating cord and passes down the hind-gut (see p. 272). Fig. 32 is of a section through the hind-gut, and shows such a mass (*fm.*) almost filling the lumen. Between it and the epithelium there can be seen a layer (*lay.*) of material which represents a mixture of finely divided food and secretion according to the above argument. It can, in fact, be seen to contain fine carmine particles (the main mass also consists of carmine), while that portion of the layer nearest to the epithelium contains blue-staining material agreeing in appearance with the vesicular inclusions of some of the cells (see p. 284). There is reason for believing that this layer is left behind while the solid mass passes backwards, for sections in front of and behind such a mass show similar layers of material; in any case, it is constantly added to as fresh material is transmitted to it from the rotating cord. It appears from fig. 32

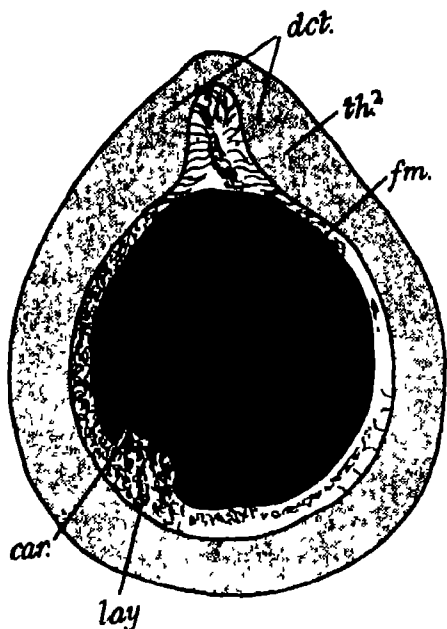


FIG. 32—Transverse section of a "faecal" mass in the hind-gut.

that addition can also be made to this material by a fragmentation of the outside of the mass as it passes on its way under the ciliary action; such a fragmentation is occurring at the point *car.*, with the release of small carmine particles. It will also be seen that above the mass there is a free space in the lumen of the hind-gut bordered by the cells of the dorsal ciliated tract (*dct.*) which laterally are taller than the cells elsewhere and thus produce a distinct thickening of the wall (*th.²*). The effect of this

is that the region of the tract is distinctly marked off from the rest of the lumen, and there is thus provided a free passage down the hind-gut for any small particles and water which are being driven down it by the dorsal tract. Here again it is difficult to judge how far this really represents the condition in the living animal, for the hind-gut does not always take up this shape in the preparations, nor is even the dorso-lateral bulge of the wall always visible; it is not seen, for example, in fig. 14, becoming visible in this particular specimen only at the extreme hind-end. However, the condition shown in fig. 32 can hardly be entirely an artefact, and it may be concluded that the dorsal ciliated tract, in addition to transmitting particles down the hind-gut and assisting in the transit of the "faecal" masses, provides also a dorsal channel which prevents complete blockage of the hind-gut during the transit of those masses. Such a device seems, in fact, functionally necessary in an animal in which a ciliary current is constantly directed backwards down the alimentary canal, and is physiologically comparable to the ventral siphon of *Balanoglossus*.

To return to the general analysis of the course of the food particles, there seems to be no necessity for a constant intake of fresh food material, a single mass of food and secretion rotating in the ilio-colon ring being sufficient to provide the animal for many hours with a supply of finely divided material for digestion and absorption. There is some reason for believing that such a condition may be the normal one; that it is a practicable one is certain, for an animal placed in a carmine suspension and subsequently restored to clean sea water may exhibit a mass of carmine in the ring for at least 24 hr. afterwards. RICE (1880) frequently found animals in which the "stomach" and "intestine" were devoid of food, and this led him to suggest that periods of feeding might alternate with periods of rest. It is also very noticeable when an animal is taking in carmine that the amount entering the mid-gut or enclosed in the food cord varies from period to period; reference has been made above to variation in the amount of carmine caught in the anterior end of the lateral ciliated tract, which would depend on variation in the amount passing down the oesophagus. It is clear that in the oral-hood cirri and the velar tentacles and velum the animal has a mechanism for controlling the quantity of particles entering with the incurrent stream of water, and in view of the above conclusions it seems physiologically possible for a period of intake of food to alternate with a period of rest, while allowing the digestion and absorption of food to proceed continuously, although how far this actually occurs under natural conditions cannot be said. Finally, it may be noted that the above account explains how absorption can take place although the "faecal masses" pass relatively quickly down the hind-gut (see p. 273), for the whole process is governed by the rotation occurring under the influence of the ilio-colon ring, and depends upon the fact that the food mass can remain for a considerable time in the latter region while particles are distributed from it over the epithelium.

8—THE INTERPRETATION OF THE MID-GUT DIVERTICULUM

The commonly implied interpretation of the mid-gut diverticulum of *Amphioxus* as homologous with the liver of the Craniates appears to be based upon the existence of an "hepatic portal system", as originally described by MÜLLER (1844), and the resemblance in the mode of development of the two organs, as described by HAMMAR (1898). The latter's argument depends upon nothing more than the fact that the diverticulum develops as a ventral groove of the mid-gut, the opening of the groove being then narrowed and the groove itself transformed into the tubular diverticulum by the backward movement of its anterior edge. It is difficult to see that this fact carries any weight at all without a great deal of confirmatory evidence. Characteristic functions of the Craniate liver are the storage of glycogen and to a less extent of fat, the regulation of urea content, and the excretion of bile pigments. With regard to the existence of the latter in *Amphioxus* it is impossible to state anything definite in the absence of experimental evidence, but their occurrence is unlikely in view of the fact that the blood is colourless. Similarly, there appears to be no evidence with regard to the nitrogen metabolism, and this aspect of the problem clearly demands investigation. However, even if the diverticulum were shown to be concerned in the regulation of urea, this would not be conclusive, for there is reason to believe that in *Scyllium canicula* the liver is not the only source of urea (NEEDHAM and NEEDHAM 1930). Evidence for the storage of fat in the diverticulum has been stated above (p. 293), but with regard to the storage of glycogen nothing definite can be said, as although a number of specimens have been fixed in Carnoy and stained with iodine, none of these has given a convincing picture of carbohydrate reserve. It may be that little is stored under natural conditions, and it may be supposed that a much more definite result would be obtained from animals artificially fed upon a carbohydrate diet; it is intended to investigate the problem further along those lines. It may be pointed out that glycogen is stored in the epithelium of the intestine of the Ascidian *Ciona* (YONGE 1925) in large glycogen cells, while similar cells have been described in the "liver" of *Cynthia* (WAGNER, quoted by SEELIGER 1893); as for this latter organ, BERRILL (1929) showed that the "liver" of *Tethyum* was a secretory organ, and he was unable to find any trace of bile salts or bile pigments in it; he did not investigate the glycogen reserves in this animal. It follows that even if the epithelium of the diverticulum of *Amphioxus* did store glycogen, it would merely be exhibiting a generalized property of the intestinal epithelium of the lower Chordates which would not strengthen the homology between the diverticulum and the Craniate liver, since such a property might be exhibited by the epithelium of any part of the gut epithelium or of any diverticulum of it. However, the occurrence of fat storage in the diverticulum provides at least some functional explanation of its blood supply, but it seems premature to call this an hepatic portal system in the absence of more extensive knowledge as to its function. On morphological grounds it is scarcely possible to justify the term,

for the description and figures of FRANZ (1927a) suggest that the network of blood vessels on the diverticulum is essentially similar to that surrounding most of the intestine, and may merely emphasize the fundamental resemblance between the two regions.

It seems clear that HAMMAR's interpretation of the diverticulum derives in part from the belief that it may be regarded as the liver of the Craniates in a simple form; FRANZ (1927a) in fact describes it as resembling that of a very young *Petromyzon* embryo. Now as a purely morphological concept this may be reasonable, but on other grounds, as the present work has shown, it is far from acceptable, for it is difficult to believe that the specialized secretory epithelium of the diverticulum could have become transformed into the totally different hepatic tissue of the Craniates. All experience suggests that the latter organ would be more likely to develop from the beginning *sui generis*.

It may be concluded so far, then, that there is as yet no sound support for the interpretation of the diverticulum as homologous with the Craniate liver, while the evidence now available tends to cast doubt upon that interpretation. It remains to enquire whether there is any other Craniate organ to which the diverticulum might be compared.

The present writer has recently directed attention to MASKELL's (1930) discovery in two Australian ammocoetes of intestinal diverticula which grow forwards from the anterior end of the intestine, at the point at which the oesophagus passes into it; two diverticula occur in *Geotria* and one in *Mordacia*. These diverticula contain presumed secretory cells with granular inclusions and single large nucleoli, and it has been shown (BARRINGTON 1936) that an accumulation of similar cells at the anterior end of the intestine in the ammocoete of *Lampetra planeri* (in which no diverticula occur) is the seat of the production of a proteolytic enzyme of the tryptic type. Now it is impossible to ignore certain resemblances between these conditions and the facts recorded above for *Amphioxus*, for in the latter there is an intestinal diverticulum which grows forward from the point of junction of the oesophagus and the intestine (mid-gut), which contains secretory cells with granular inclusions and single large nucleoli, and from which digestive enzymes, including a protease of a tryptic type, can be extracted. That the cells in question are comparable in the two forms seems reasonably clear; in *Amphioxus*, however, they occur also in the mid-gut, cells of a similar appearance being conspicuous in the hind-gut as well. This scattered distribution makes it impossible to correlate them with the production of the protease as clearly as in the ammocoete where they are very localized, although the proteolytic activity of the mid-gut and hind-gut extracts would be in agreement with that correlation.

It is possible to regard the granular cells in the ammocoete as representing zymogen cells which have become massed together prior to separation as a pancreas, while the development of the diverticulum in the Australian forms would indicate a further step in that direction (for a discussion, see BARRINGTON 1936). If this comparison

should prove well founded, it would be possible to compare the diverticulum of *Amphioxus* with the pancreas of the Craniata at least as plausibly as with the liver. This is not to suggest that the diverticulum of the ammocoete or the pancreas of the higher Craniata actually evolved directly from the diverticulum of a Protochordate ancestor, or that the diverticulum of *Amphioxus* should be called a pancreas, for the method of functioning of its digestive system is so different from that of a typical Craniate that any such direct homology seems likely to be unsound. It is sufficient to assume that in the lower Chordates there is a tendency for the intestine to grow forwards as a diverticulum from a point just behind the oesophagus. In *Amphioxus* this tendency provides for the development of a secretory organ advantageous in an animal with a very short alimentary canal, as well as perhaps a storage organ, while in the Craniata it results eventually in the development of a pancreas, associated with the increasing concentration of the granular type of zymogen cell which at the lower evolutionary level of *Amphioxus* has a more scattered distribution. It would further seem likely, according to this hypothesis, that the "liver" in the Tunicata is homologous with these diverticula, but unfortunately it is not yet established that secretory cells with large nucleoli occur in this organ. BERRILL (1929) showed that the "liver" of *Tethyum* produced a brownish secretion comprising a variety of digestive enzymes, and was unable to find in it any indication of the presence of bile salts, bile pigments or cholesterol, but he does not refer to the existence, in that organ or elsewhere in the gut, of secretory cells characterized by the possession of large nucleoli. It is all the more important, therefore, that YONGE (1925) has briefly described cells which satisfy that definition in the epithelium of the gut of *Ciona*; this form, however, unfortunately lacks a "liver". In view of this discrepancy between the secretory cells of *Tethyum* and of *Ciona*, it is proposed to investigate further the histology of the gut of the Ascidians.

Finally, it must be emphasized that the above hypothesis as to the homology of the diverticulum of *Amphioxus* is not final, for further work is clearly necessary before any definite conclusions can be drawn. Some at least of this work it is hoped to undertake in the immediate future. In the meantime, nothing more is claimed than that the new interpretation is at least as plausible as the earlier suggestion of homology with the Craniate liver, and that, so far as the cytological and functional observations recorded during the present work are concerned, it appears to be based upon a rather more secure foundation.

9—SUMMARY

An account is given of the morphology, cytological structure and ciliary mechanisms of the post-pharyngeal region of the alimentary system of *Amphioxus*, with a description of the enzymes.

Two types of secretory cell occur in the mid-gut and mid-gut diverticulum, one with

granular inclusions and a large nucleolus and the other with vesicular inclusions and a small nucleolus, the nucleus in the latter type being more granular. Similar (and possibly identical) cells occur in the hind-gut. The first type of cell recalls the zymogen cells of the anterior end of the intestine of ammocoetes.

A third type of cell without inclusions and with a slender and densely staining nucleus is found in the ilio-colon ring, and is associated also with the formation of localized ciliary tracts. The cilia occurring elsewhere are less active than the cilia of these cells. These tracts consist of a very conspicuous tract on the left wall of the anterior region of the mid-gut, for which the name of 'lateral ciliated tract' is proposed, and a dorsal tract, continuous with the preceding, which extends from the posterior half of the mid-gut to the hind end of the hind-gut, and for which the name of "dorsal ciliated tract" is proposed.

Absorption occurs in the hind-gut and to some extent in the hinder portion of the mid-gut; there is a possibility of some absorption occurring in the diverticulum, but the appearance of artificial food material in the cells of this region is considered to be largely due to transport in the blood stream. Solid material is ingested, and digestion is believed to be completed within the cells after preliminary extracellular action.

It is shown that starch, glycogen, sucrose, salicin, maltose, lactose, triacetin, gelatin and casein can be digested, but not inulin, sawdust or gum arabic. Digestive activity is shown by extracts of the diverticulum, mid-gut and hind-gut, but not of the pharynx. Some indication of the pH range of the amylase, lipase and protease is given.

Ciliary currents in the diverticulum drive its secretions downwards and backwards into the mid-gut. Here they and the secretions of the mid-gut are mixed with the food cord which is set into rotation by the powerful ciliation of the ilio-colon ring. The lateral ciliated tract adds to this rotating mass material which is swept down the wall of the oesophagus outside the main food cord.

Particles of food mixed with digestive secretions are broken off from the rotating mass and are distributed over the absorptive area by the backwardly directed currents of the dorsal ciliated tract, the ilio-colon ring and part of the left wall of the anterior end of the hind-gut. Material can also be broken off from the "faecal" masses as they pass down the hind-gut after becoming separated from the rotating mass. Particles which are driven forwards in the mid-gut under the influence of reverse eddies are restored to the main mass by the lateral ciliated tract. The main food cord does not enter the diverticulum, and only small quantities of scattered particles can enter this organ, the general trend of the ciliary currents being directed away from it.

The homology of the mid-gut diverticulum is discussed in the light of the present work. Reasons are given for regarding its alleged homology with the liver of the Craniata as insecurely founded, and it is suggested as an alternative that it may be homologous with the intestinal diverticula of certain ammocoetes, and possibly, through them, with the exocrine component of the pancreas of the Craniata, these

organs arising as a result of a common tendency in the lower Chordata for the development of one or more diverticula at the anterior end of the "intestine".

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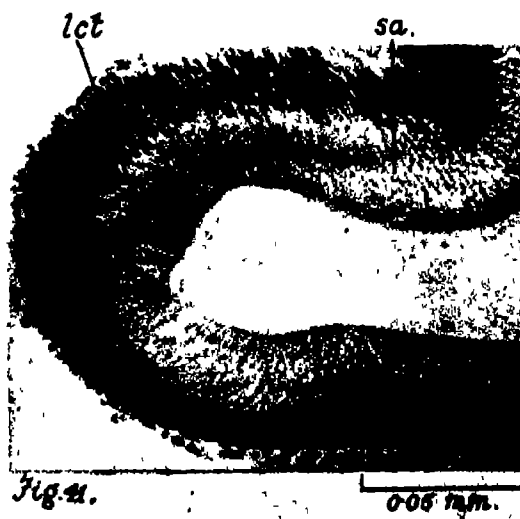
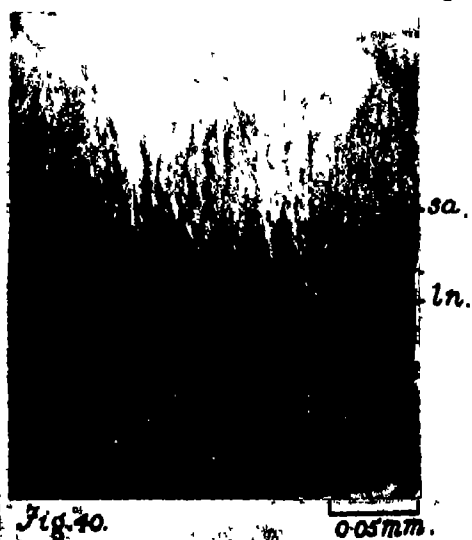
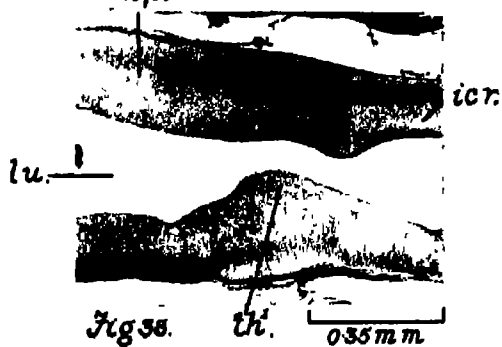
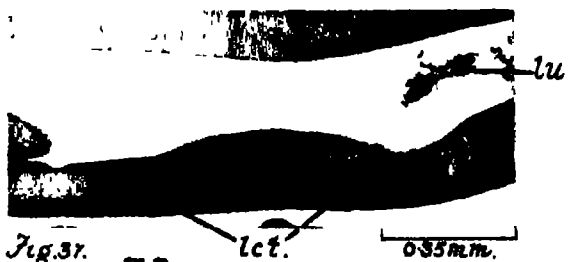
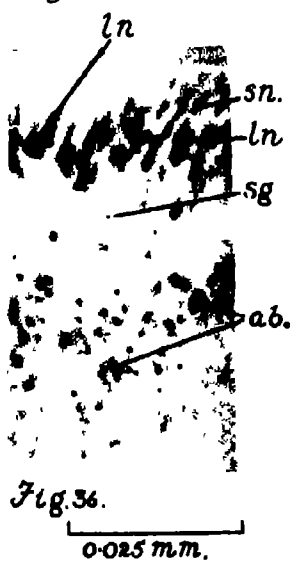
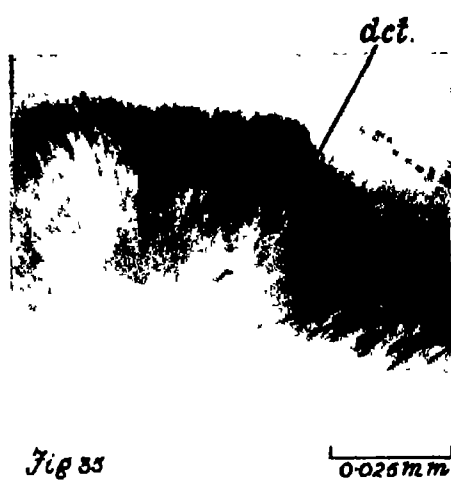
EXPLANATION OF LETTERING

<i>ab.</i>	inclusions, presumed products of absorption	<i>cr.</i>	strongly ciliated cells of the ilio-colon ring
<i>an</i>	anus	<i>c.oes.</i>	cells of the oesophageal type
<i>ant.</i>	anterior	<i>dc.</i>	dorsal current of the diverticulum
<i>car.</i>	caruncle particles	<i>dct.</i>	dorsal ciliated tract
<i>ced.</i>	circular eddies	<i>div.</i>	diverticulum
<i>ch</i>	strongly ciliated cells of the hind-gut	<i>fc.</i>	food cord
<i>cl.</i>	clear area	<i>fm.</i>	"faecal" mass
		<i>hg.</i>	hind-gut

<i>icr.</i>	ilio-colon ring	<i>par.</i>	particles
<i>lat.</i>	lateral wall	<i>ph.</i>	pharynx
<i>lay.</i>	layer of food material and secretion	<i>post.</i>	posterior
<i>let.</i>	lateral ciliated tract	<i>r.</i>	dark band
<i>lh.</i>	less strongly ciliated cells of the hind-gut	<i>sa.</i>	secretory cells, type A
<i>ln.</i>	large nucleolus	<i>sb.</i>	secretory cells, type B
<i>lu.</i>	lumen of mid-gut	<i>sec.</i>	secretory epithelium
<i>ma.</i>	mid-gut (anterior)	<i>sg.</i>	secretory granules
<i>md.</i>	mid-dorsal line	<i>sn.</i>	small nucleolus
<i>mp.</i>	mid-gut (posterior)	<i>s-z.</i>	reference points
<i>mv.</i>	mid-ventral line	<i>tb.</i>	transverse band
<i>my.</i>	cut edge of myomeres	<i>th.^{1,2}</i>	thickening of epithelium
<i>oes.</i>	oesophagus		

PLATE 28

- FIG. 33—Transverse section of the dorsal ciliated tract in the ilio-colon ring (haematoxylin)
- FIG. 34—Transverse section of the epithelium in the mid-gut diverticulum (Mallory).
- FIG. 35—Transverse section of the dorsal ciliated tract in the mid-gut (haematoxylin).
- FIG. 36—Transverse section of the epithelium in the hind-gut (haematoxylin).
- FIG. 37—Horizontal section of the lateral ciliated tract (Mallory).
- FIG. 38—Horizontal section of the "sphincter" between the mid-gut and the ilio-colon ring (Mallory).
- FIG. 39—Transverse section of the epithelium in the mid-gut diverticulum (haematoxylin).
- FIG. 40—Transverse section of the epithelium in the posterior half of the mid-gut (Mallory).
- FIG. 41—Transverse section of the mid-gut diverticulum, passing through the anterior end of the lateral ciliated tract (haematoxylin).



VII—THE WOOD ANATOMY OF THE FAMILY STERCULIACEAE

BY M. M. CHATTAWAY, M.A., B.Sc., D.Phil.,

*Imperial Forestry Institute, Oxford**(Communicated by R. S. Troup, F.R.S.—Received 8 April, Read 17 June 1937)*

[Plates 29–31]

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I—INTRODUCTION

The group Malvales is moderately well defined, both taxonomically and in the anatomy of the wood, but, to judge by the frequency with which the limits of the families are constantly being revised, its division into families and subfamilies is still a matter of some difficulty to taxonomists. A similar state of affairs exists in regard to the wood, for, although it is comparatively easy to recognize a specimen as one of the Malvales, it is often extremely difficult to decide to which family it belongs. This is possibly due in large part to faults in the systematic grouping, and the present investigation was undertaken to find out whether the groups suggested by the wood anatomy can be used to clarify the taxonomic position. In one family in particular, the Sterculiaceae, the wood anatomy suggests a heterogeneous group that needs revising, and this family has therefore been selected for detailed study. Two aims have been kept in view: the first, to find out how far the wood anatomy is in agreement with the present taxonomic classification, and what changes it suggests; the second, to try to ascertain which features in the wood are peculiar to a family, and imply relationship, and which are to be encountered in unrelated families, and represent stages in phylogenetic development.

The need for a revision of the existing classification of the family became evident at an early stage in the investigation, for the family Sterculiaceae, as established by

BENTHAM and HOOKER (1867), includes a group of woods with a very wide range of structure; and, in spite of having given its name to the family, the genus *Sterculia* is by no means typical of it. Further investigation led to the conclusion put forward by the author (CHATTAWAY 1932), that not only is *Sterculia* not typical of the family as a whole, but that the entire subfamily to which it belongs differs considerably from all the other subfamilies of BENTHAM and HOOKER's Sterculiaceae. The anatomical differences are indeed so great that it seems inappropriate to retain for so large a family as the one they delimited a name that is associated with an isolated group within it. This conclusion, based upon anatomical characters, supports that which EDLIN (1935) drew from the floristic characters, namely, that the family name should be restricted to the Sterculieae. In the past, various authorities have suggested that this subfamily stands apart from the others, but none of them raised it to the status of a separate family, although, as EDLIN states, the differences between the Sterculieae and the related subfamilies (under the old classification) are quite as sharp as those separating many other families. This idea was indeed put forward by DUMONT (1887), who concludes from the general anatomy that the Sterculieae is "a group parallel to the primary families Malvaceae, Bombacaceae and Tiliaceae".

Within the subfamily Sterculieae itself there has been further confusion in the classification of the genera. The genus *Sterculia* has often been reclassified and redivided; for example, the genera *Brachychiton*, *Ebriobroma*, *Firmiana*, *Pterygota*, *Pterocymbium* and *Scaphium* have all at one time or another been classified as *Sterculia*. EDLIN (1935) is unable to find any satisfactory means of distinguishing these genera floristically, and states (p. 9) that within the tribe Sterculineae the *Colas*, confined to Africa, are not easily distinguished from the more widely distributed *Sterculias*, and, with the exception of *Tetradia* and *Octolobus*, the other genera have all at one time or another been accounted *Sterculias*. He therefore divides the family into two tribes, containing only seven genera, as follows:

STERCULINEAE: *Sterculia* L.; *Cola* SCHOTT; *Tetradia* R.BR.; *Octolobus* WELW.

TARRIETINEAE: *Tarrietia* BL.; *Heritiera* AIT.; *Argyrodendron* F. VON MUELI.

EDLIN concludes, "until this group, numbering at least 150 species scattered throughout Asia, Africa, Australia and South America, has been revised monographically by one investigator, it will be impossible to say with finality what genus should or should not stand....It is possible that a careful consideration of the wood structure of the Sterculiaceae may throw light on the value of the generic divisions". The present author has made such a study, and has been able to suggest changes in regard to some of the difficult genera.

The second aim of the investigation is to study the relative values of the anatomical characters that are commonly used for diagnostic purposes, by examining their occurrence within a group of closely allied woods. Research by other workers upon

vessel-member length (FROST 1930*a*, *b*), pitting (FROST 1931) and ray type (KRIBBS 1935), has established a sequence of development for each of these features, so that it is possible to say whether a wood is primitive or advanced in regard to them. By using these established characters it is possible to decide upon the level of development attained by any family, such as the Sterculiaceae, and also to arrange the genera within the family in order, representing an advancing series of specialization in regard to vessel-member length, pitting or ray type. The evidence obtained from such a series can then be used to test the other features in the wood. This method has been applied to the Sterculiaceae, for vessel diameter, vessel number, the percentage of solitary vessels, fibre length and the type of parenchyma, but the conclusions that are drawn can only be applied within this family. Probably some of the developmental series will be found to apply only to single families or groups of families, and to have no general application, while others apply to all woods, irrespective of their family. The former may imply relationship, the latter must be regarded as "stage features" (CHALK 1935), representing stages of development passed through by members of totally unrelated groups. The great difficulty in this part of the work has been that the anatomist must use the genera, as established by the taxonomists, to find out what are reliable generic characters, while, as will be shown in § XII, the taxonomists are not unanimous as to the constitution of the different genera.

In handling the material assembled for this investigation two alternative methods of presenting the facts were considered. The first, that of treating each genus separately and then reviewing the anatomical characteristics of the family as a whole, was discarded; it would involve much repetition, because the genera are very much alike in several of the features examined. The second method, which is the one adopted, is to consider each anatomical feature separately, establishing so far as possible the position of each genus in regard to that feature, and finally to give an abbreviated description of the genera, picking out the particular features by which each can be distinguished from the rest. The position of the genera is discussed and the proposed changes explained and justified in § XII. By this means the material relating to the systematic position of the genera can be placed together and can be read without the necessity of disinterring it from a mass of anatomical detail. Similarly, each separate anatomical feature has as far as possible been treated comprehensively in the appropriate section, so that the facts relating to any particular tissue can be found together.

II—TERMINOLOGY

The technical terms used in this paper are for the most part those approved by the International Association of Wood Anatomists, and published in the "Glossary of Terms used in Describing Woods" (1933). Where these terms are new, or differ from those previously in use, they are explained in footnotes.

III—MATERIAL AND METHODS

The material examined is as follows (the species are given in § XII):

<i>Argyrodendron</i> F. VON MUELL.	17 specimens of	3 species.
<i>Brachychiton</i> SCHOTT and ENDL.	10 „	3 „
<i>Cola</i> SCHOTT	29 „	13 „
<i>Eribroma</i> PIERRE	1 „	1 „
<i>Firmiana</i> MARSIGLI	9 „	5 „
<i>Heritiera</i> AIT.	16 „	4 „
<i>Octolobus</i> WELW.	1 „	1 „
<i>Pterocymbium</i> R.BR.	9 „	2 „
<i>Pterygota</i> ENDL.	7 „	3 „
<i>Scaphium</i> ENDL.	7 „	4 „
<i>Sterculia</i> L.	72 „	32 „
<i>Tarrutia</i> BL.	27 „	7 „

In all 205 specimens of seventy-eight species, representing twelve different genera, were examined. Of these *Cola*, *Eribroma* and *Octolobus* are confined to tropical Africa, and *Brachychiton* and *Argyrodendron* to Australia; *Scaphium*, *Pterygota*, *Firmiana* and *Pterocymbium* occur in tropical Asia; *Tarrietia* occurs in tropical Asia and Australia, and *Heritiera* in tropical Asia and tropical Africa; *Sterculia* has a very wide range of distribution throughout the entire tropics. *Tetradia* is now extremely rare, and it has not been possible to obtain material of this genus.

Transverse, tangential and radial sections of the wood, cut at 10–15 μ , have been used for the general investigation of the family, but special sections, cut at about 5 μ and mounted in glycerine jelly, were used for the investigations of the pitting, and sections treated with various solvents for the investigation of the crystals.

A special technique was used for the study of the rays, in order to follow their development from the cambium, and to trace the changes that take place in the cambial initials. The method that has been employed here is similar to that by which BEIJER (1927), KLINKEN (1914) and NEEF (1920) studied the structure of stratified cambium, and is a modification of that which ZIJLSTRA (1909) employed in his macroscopic study of ray structure. A wedge of wood, extending if possible from the pith to the cambium, was cut out with each side parallel to the rays, and deep enough to contain the tallest ray. Serial tangential sections were cut at 30 μ ; this distance was determined by the radial dimensions of the ray cells, so as to ensure that each ray cell should be represented on at least one section. By following these sections from the pith towards the periphery a picture of the cambium could be obtained as it was at different stages of development.

IV—MEASUREMENTS

Only three features have been measured for the purposes of this paper, fibre length and vessel-member* length, both measured on slides of macerated material, and vessel diameter, measured from transverse sections.

Fibre length involves little difficulty in measuring, except that of avoiding broken fibres, and of distinguishing between libriform fibres and fusiform wood parenchyma cells† in woods like *Pterocymbium* sp. and *Sterculia crassiramea* MERRIL.

Vessel-member length has been measured on macerated material, as advocated by CHALK and CHATTAWAY (1934), and the measurements were made from tip to tip of the vessel member and not from perforation plate‡ to perforation plate as before. For this reason the figures given here are occasionally slightly higher than those given in the earlier paper (CHATTAWAY 1931), because, although "tails" are exceptional in woods such as these with almost horizontal perforation plates and marked storeys, there is often a tendency for the vessel to take a lateral rather than a vertical course, and for the perforation plate to be formed in the side wall rather than in the end wall of the vessel member (CHALK and CHATTAWAY 1934).

In measuring vessel diameter, only the tangential diameter has been used. The committee of the International Association of Wood Anatomists has not yet issued any definite recommendation in regard to vessel diameter, and the terms of size used here are those proposed by CHATTAWAY (1932). It is, however, very likely that when more data have been collected from special investigations such as this, the classes may need considerable readjustment. Formerly only solitary vessels were measured, but for this investigation all the vessels have been measured, and, although this increases the range of size in any one sample, especially in woods with groups and clusters of vessels, it probably gives a truer picture of the wood than the method previously used.

Variation within a single species always presents a difficulty, and it is unwise to base the mean value for any feature on less than five or six samples from different trees. Measurements alone are seldom sufficient to distinguish between the different species of a genus even where the mean is based upon adequate material. The measurements used in this paper are expressed as the mean of each genus, and the range of the different samples is indicated by plus and minus twice the standard deviations of the means. Measurements from immature wood have been excluded so far as possible.

In a recent issue of "Trees and Timbers of the British Empire" (CHALK, CHATTAWAY, BURTT DAVY, LAUGHTON and SCOTT 1935), a new method of expressing the mean

* *Vessel member or vessel element*: one of the cellular components of a vessel.—To replace *vessel segment*. Further use of the term *segment* is discouraged, since it implies the reverse of the actual process of vessel formation.

† *Fusiform wood parenchyma cell*: a wood parenchyma cell derived from a cambial initial without subdivision.—To replace *substitute* and *intermediate wood fibre*, which are inappropriate since they obscure the fact that the cells are parenchyma.

‡ *Perforation plate*: the area of the wall (originally imperforate) involved in the coalescence of two members of a vessel.

and range of a genus diagrammatically was used, and the same method is employed here.

Vessel number was, so far as possible, counted on 25 sq. mm., but occasionally, where only a small block was available for sectioning, a smaller area was used; in every case complete growth periods were covered so as to include vessels from both early and late wood.

V—STOREYED STRUCTURE

The genera of the Sterculiaceae are all characterized by regular storeyed structure, which produces distinct ripple marks on the tangential surface of the wood. Storeyed structure only occurs in rather highly specialized woods which have short cambial initials, and is due to the method of multiplication of the cambial initials, by longitudinal instead of by transverse divisions (BAILEY 1923; BEIJER 1927). This regular arrangement of the initials is reflected in the tissues formed from them, affecting the shape of the vessels, fibres and parenchyma strands (CHALK and CHATTAWAY 1934, 1935; JANSSONIUS 1931). A further result of this method of division is that the range of length in the vessel members decreases. CHALK and CHATTAWAY (1935) investigated this, and showed that in storeyed woods the shortest vessel member is usually more than half the length of the longest, while in non-storeyed woods it is always less than half. This affects the coefficient of variation (RENDLE and CLARKE 1934); for non-storeyed woods it was found to be 0.188, and for storeyed woods 0.111. In the Sterculiaceae the storeys are very regular, and the range in cambial initial length within the family and the coefficient of variation (0.102) are very small. The higher coefficient of variation for storeyed woods generally is due to the inclusion of woods in which both methods of division occur. The marked regularity of the storeys in all the genera of the Sterculiaceae and the low coefficient of variation suggest that in this family transverse or oblique divisions of the cambial initials are uncommon.

As storeyed structure is due to short cambial initials it indicates that the woods are advanced, but apart from this it has no phylogenetic significance. It is, however, a very useful diagnostic feature, and the height of the storeys provides a simple means of estimating the length of the cambial initials. If the rays are short they too may be storeyed, and this combination of features is of still further use in recognizing woods. In the Sterculiaceae, the uniseriate rays are often storeyed, but, owing to their peculiar method of growth, the large rays are often quite independent of the storeys.

VI—VESSELS

(a) *Vessel-Member Length*

The study of vessel-member length throughout a large number of families has shown (BAILEY 1920) that the length of a vessel member is approximately the same as that of the cambial initial from which it was derived, and the significance of vessel-

member length lies in the fact that it provides a measure of the length of the cambial initial where the initials themselves are not available. In hardwoods generally a wide range in vessel-member length may be encountered, and FROST (1930*a, b*, 1931) has shown that there is a relation between the length of the vessel members, the type of perforation plate and the type of intervacular pitting. He has also established the fact that, on the whole, woods with long vessel members, scalariform perforation plates and scalariform intervacular pitting are more primitive than those with shorter vessel members, simple perforation plates and alternate intervacular pitting. Subsequent research has confirmed his conclusions, and it is now possible to use vessel-member length to deduce the relative positions of woods from different families; and also as a guide to the relative position of the genera within a family, and to assess the significance of other features in the wood.

The vessel-member length for the different genera in the Sterculiaceae is shown in fig. 1. There is considerable overlapping between the ranges for the different genera

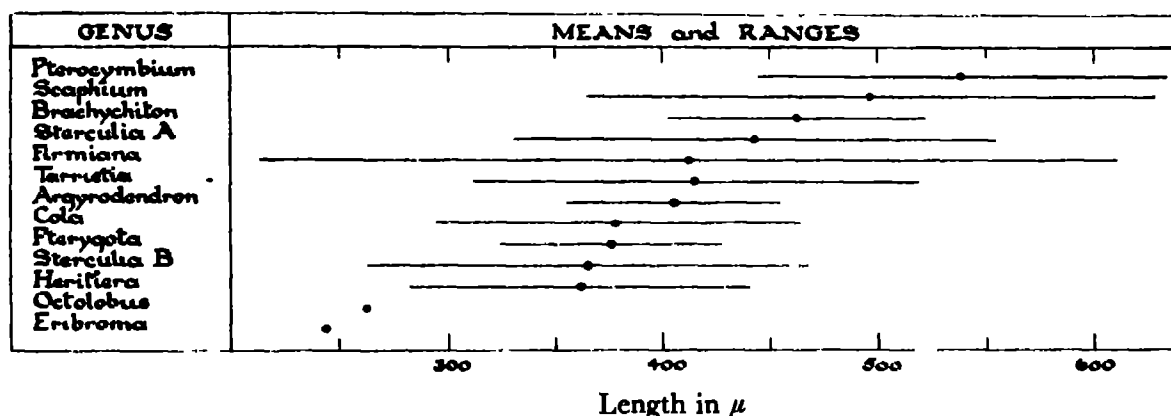


FIG. 1.—Diagram to show vessel-member length in the Sterculiaceae.

(especially for *Firmiana*), but nevertheless there is a significant* difference between the values at the top and bottom of the series. The means were based on all the named species of each genus available, but in some cases this was not very many, and the means might be liable to some slight alteration if more material were available.

It will be seen that the genus *Sterculia* appears twice in this list. A greater difference in structure was found between some of the species of this genus than between some of the other genera, and the *Sterculia* species were accordingly separated into two groups, one with narrow lines of parenchyma and one with broad bands of parenchyma. The former is referred to as *Sterculia* A, the latter as *Sterculia* B. The difference in vessel-member length between the two groups appears to be significant.

Following FROST's (1930*a, b*) assumption that the more primitive woods have the

* Calculated from the formula $Ed = \sqrt{(Ea^2 + Eb^2)}$, where Ed = the standard error of the difference and Ea and Eb the standard errors of the means compared. To be significant the difference between the means should be more than three times the standard error of the difference.

longest vessel members, there is some likelihood that the woods at the top of the series given in fig. 1 are slightly more primitive than those at the bottom of the series, although there is so little range throughout the whole family that no extreme differences would be expected. Furthermore, although it is quite possible that further material of any genus might alter its mean to some extent, and that changes in position between adjacent members of the series might be necessitated, it is nevertheless very unlikely that further measurements would cause changes in position between the top and bottom of the series, or between widely separated members of the series. The series into which the genera may be arranged for any one feature is not intended to be regarded as fixed or unalterable, especially when it is based upon measurements, but rather as indicating the general trend of development in that feature.

From a consideration of vessel-member length it seems probable that woods such as *Pterocymbium*, *Scaphium*, *Brachychiton* and *Sterculia* A might be expected to be more primitive than *Cola*, *Pterygota*, *Heritiera* and *Sterculia* B; but the range met with in the different genera suggests that the family as a whole is a specialized one, and the mean vessel-member length of the entire family is short ($398\mu \pm 80.5$).

(b) Vessel Diameter

Unfortunately very little research has yet been done upon the significance of vessel diameter, but BROWN (1921) and FROST (1930a) have both suggested that vessels tend to be smaller in primitive woods than in specialized ones. CHALK and CHATTAWAY (1935) found no general relation between vessel diameter and vessel-member length, though they found that the maximum widths recorded all occurred in woods with relatively short vessel members. The woods measured by these authors were classified according to the type of perforation plate and the presence or absence of storeyed structure, and it was then found that within a limited range of length, such as occurs in any of these groups, the longest vessel members tend to be broader than the shortest.

The vessels in the Sterculiaceae are usually of moderate size; small vessels occur in a few species of *Cola* and in *Octolobus*, and rather large ones in *Pterocymbium* and some species of *Sterculia*. The range in size within any one sample is often very great, especially in woods that have a large proportion of clusters and groups of vessels. In these woods there may be, within a single group, vessels varying from 30 to 200μ in tangential diameter.

The means and ranges for the different genera are shown diagrammatically in fig. 2. Excluding *Octolobus*, of which only one sample was available, there is only 84μ difference between the largest and the smallest mean, and the ranges overlap very much. Nevertheless the difference between both *Cola* and *Brachychiton* at the bottom of the series and *Pterocymbium* at the top appears to be significant. *Brachychiton* and *Sterculia* A are very much alike in all other features, and the vessels of *Brachychiton* are unexpectedly small.

The chief interest in this series lies in the support it gives to the conclusions of CHALK and CHATTAWAY (1935) mentioned above. The woods of the genera of the Sterculiaceae form a series within the limits of vessel-member length associated with storeyed structure, similar to those examined before, but not including any of the same genera. The correlation coefficient between vessel-member length and vessel diameter for these woods is $+0.655 \pm 0.107$, but the relation is probably masked to some extent by the relations that may exist between these two features and the percentage of solitary vessels and the number of vessels. This will be further discussed at the end of this section.

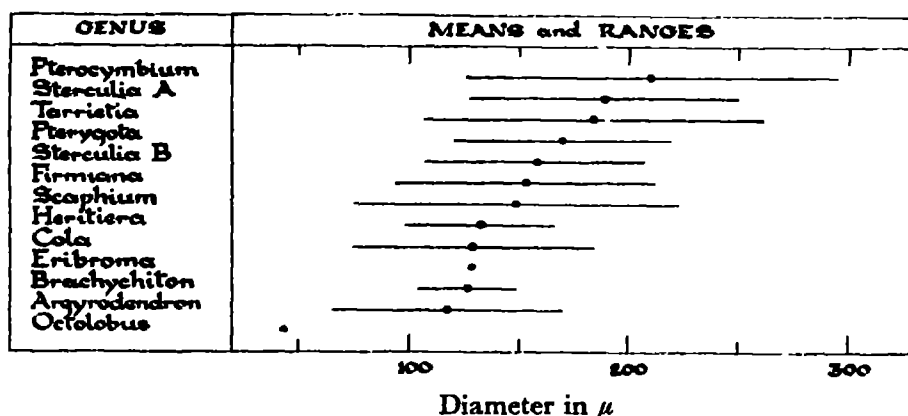


FIG. 2—Diagram to show vessel diameter in the Sterculiaceae.

(c) Distribution

The author was unable to find any evidence from the work of other investigators for assigning any phylogenetic significance to vessel number, and the results given below show that within this group the number of vessels is not significantly related to the degree of specialization of the wood as inferred from the length of the cambial initials. The extreme variability of vessel number under the influence of external conditions renders it a somewhat unsatisfactory feature to work with, and there appears to be great variation, not only between the different samples of the same species, but occasionally even in different parts of the same sample. In spite of this, however, it is possible to arrive at an estimate that is sufficiently accurate to show whether the vessels are few or numerous, solitary or grouped.

The vessels in the Sterculiaceae are never numerous, and the entire range encountered was only 1–13 sq. mm. There is only a slight difference in vessel number between the different genera, but there is a difference between the two subfamilies. In the Sterculineae the vessels are (with the exception of *Octolobus*) fewer than in the Tarrietineae.

The relative frequency of solitary vessels and vessel groups was computed at the same time as vessel number; the results of both counts are shown in Table I.

The high percentage of solitary vessels in *Pterocymbium*, *Sterculia* A and *Tarrietia* are useful in helping to distinguish the woods of these genera, and the difference in the percentage of solitary vessels between *Sterculia* A and *Sterculia* B serves to emphasize the difference in structure between the two sections of the genus.

A study of the figures obtained for vessel diameter, vessel-member length, the number of vessels per unit area and the percentage of solitary vessels suggested that

TABLE I—THE PERCENTAGE OF SOLITARY VESSELS AND THE NUMBER OF VESSELS PER SQ. MM.

Genus	% of solitary vessels		No. of vessels per sq. mm.	
	Mean of means	S.D. of means	Mean of means	S.D. of means
<i>Pterocymbium</i>	65.9	± 14.6	1.2	± 0.50
<i>Sterculia</i> A	63.2	± 14.5	3.2	± 1.58
<i>Tarrietia</i>	53.0	± 15.5	5.2	± 1.71
<i>Scaphium</i>	44.6	± 17.1	2.4	± 1.06
<i>Firmiana</i>	41.4	± 16.5	3.2	± 1.04
<i>Eribroma</i>	41.0		1.4	
<i>Brachychiton</i>	40.9	± 9.7	3.2	± 0.91
<i>Herniera</i>	39.2	± 14.3	5.1	± 1.83
<i>Pterygota</i>	38.9	± 19.0	2.6	± 1.69
<i>Cola</i>	35.4	± 15.7	3.1	± 1.68
<i>Sterculia</i> B	32.3	± 14.6	2.5	± 0.99
<i>Octolobus</i>	25.0		11.0	
<i>Argyrodendron</i>	20.7	± 11.4	4.7	± 2.09

these features are to some extent interdependent, and that correlation coefficients between any two of them may be misleading. The method of partial correlation coefficients was therefore adopted. The primary purpose of these measurements was to find out whether the size and distribution of the vessels is in any way related to their degree of specialization, as indicated by the vessel-member length. It was observed that the vessels tended to be more solitary in the woods with long vessel members, and a correlation coefficient between vessel-member length and the percentage of solitary vessels of $+0.564 \pm 0.127$ confirmed this impression. But this relation disappears if a partial correlation coefficient (-0.062) is calculated, eliminating vessel number and diameter. It is suggested therefore that the apparent relation between vessel-member length and the percentage of solitary vessels can be traced to diameter. For example, within this group, woods with long vessel members tend to have wide vessels, and wide vessels are characteristically solitary. But specialization of the wood appears to have no direct relation to the percentage of solitary vessels. Similarly it was found that there is no relation between the degree of specialization and the number of vessels. There does, however, appear to be a relation between specialization of the wood and the diameter of the vessels; such a relation has already been indicated (BROWN 1921; CHALK and CHATTAWAY 1935; FROST 1930a). In the Sterculiaceae the simple correlation between these features was $+0.655 \pm 0.107$; the partial coefficient, eliminating the percentage of solitary vessels and vessel number, was rather lower ($+0.408$).

One striking relation emerged from these figures—between the diameter and the percentage of solitary vessels. The simple correlation coefficient was $+0.922 \pm 0.028$, which has increased to $+0.938$ in the partial correlation coefficient eliminating vessel-member length and vessel number. This relation does not appear to have any significance beyond the fact that where groups are common the number of small vessels is increased, and the mean diameter correspondingly reduced.

Making use of FROST's (1930*a, b*) conclusions regarding vessel-member length, one is led to the conclusion that *Pterocymbium* is the most primitive genus, and that, allowing for some slight interchange in position between the genera, there is a gradual progressive specialization through a series running as follows: *Pterocymbium*, *Scaphium*, *Brachychiton*, *Sterculia* A, *Firmiana*, *Tarrietia*, *Argyrodendron*, *Cola*, *Pterygota*, *Sterculia* B, *Heritiera*, *Octolobus*, *Eribroma*.

Specialization of the wood within this group of genera does not seem to be related to the distribution of the vessels, but there is a slight positive relation to diameter.

VII—PITTING

Research by FROST (1929) upon the pitting of dicotyledonous woods has shown that the shortening of the vessel members that indicates phylogenetic specialization is accompanied by changes in the sculpturing of the lateral walls, and that there is a parallel sequence of types of pitting from scalariform, through opposite to alternate arrangement. Alternate pits are usually associated with simple perforation plates, and are probably characteristic of the majority of dicotyledonous woods. Within this large group of woods there is considerable variation in the shape and size of the pitting, but no attempt appears to have been made to study the significance of these variations.

In the majority of woods with alternate pitting the intervascular pit-pairs* are arranged in such a way that number and size are complementary features. In a few woods the pit-pairs are widely and irregularly spaced, but in most woods the pits are so close together and so regular that their size can be conveniently estimated by the number in a given area. Using this method of estimating size the genera in the Sterculiaceae have been arranged in three groups, according to the size of their intervascular pit-pairs:

Group I: 3–6 pits per $275\mu^2$. *Brachychiton*, *Pterocymbium*, *Pterygota*, *Sterculia* A.

Group II: 6–10 pits per $275\mu^2$. *Cola*, *Eribroma*, *Firmiana*, *Scaphium*, *Sterculia* B (except *S. quinqueloba* and *S. coccinea*), *Tarrietia*.

Group III: 10–16 pits per $275\mu^2$. *Argyrodendron*, *Heritiera*, *Octolobus*.

The genus *Sterculia* occurs in two groups; in the species included in *Sterculia* A the pits are few and large, and are very similar to those of *Brachychiton*; in *Sterculia* B they

* Pit-pair: two complementary pits of adjacent cells.

are smaller and more numerous, like those of *Cola*; the only exceptions to this are *S. quinqueloba* K. SCHUM. and *S. coccinea* ROXB., which have larger and fewer pits than would be expected, and show the type of pitting otherwise restricted to *Sterculia* A.

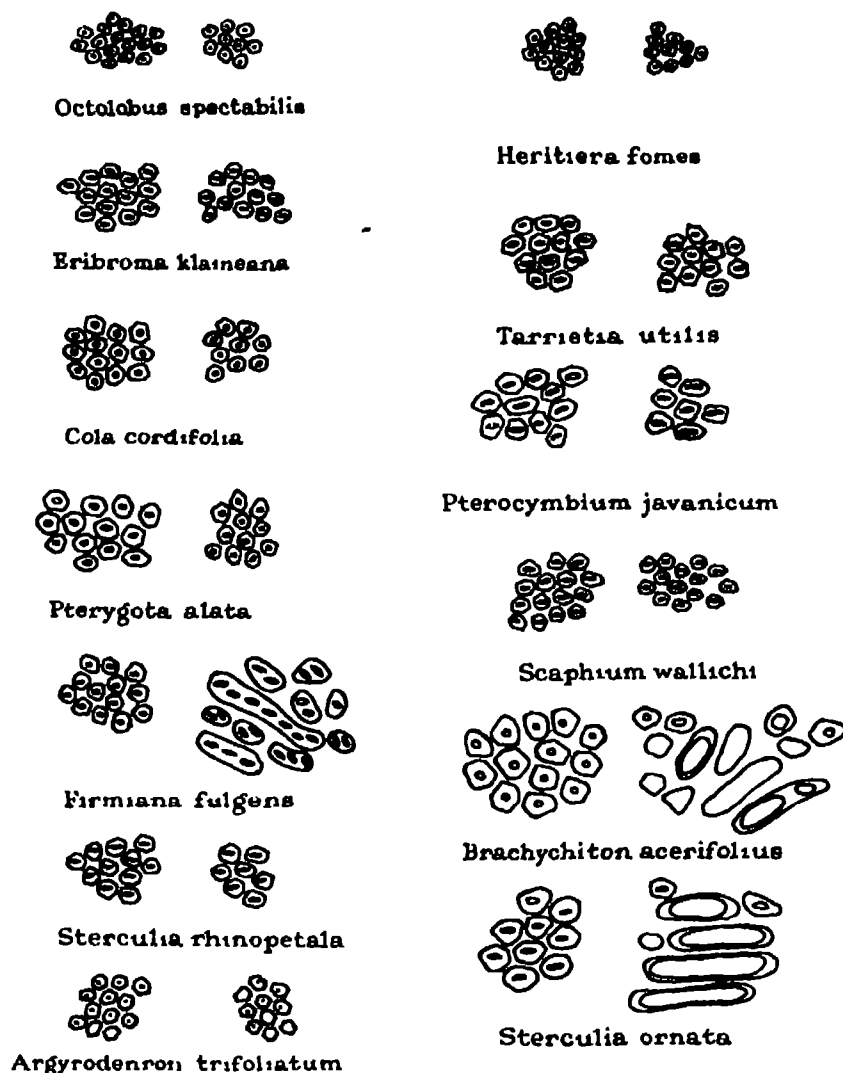


FIG. 3—Intervascular and vessel-parenchyma pit-pairs. In each pair the intervacular pitting is on the left and the vessel-parenchyma pitting on the right. (\times approx. 500.)

Fig. 3 illustrates some of the types of vascular pitting in the different genera. In each case the group of pits on the left represents pits to other vessels, and the group on the right pits to parenchyma cells. The difference in size between the intervacular pit-pairs in *Sterculia ornata* WALL. (*Sterculia* A) and *S. rhinopetala* K. SCHUM. (*Sterculia* B) is clearly shown.

The pits to other vessels are always alternate, commonly hexagonal in outline, and

the apertures are round or elliptical, and frequently coalescent.* Fig. 4 illustrates two intervascular pit-pairs, similar to one another in shape, but with coalescent apertures towards one vessel. These coalescent apertures occur where the thickness of the wall between the pits is less than the thickness of the rest of the vessel wall. Drawings of similar pits in *Sterculia quinqueloba* K. SCHUM. are illustrated in fig. 5. Fig. 5 (1) corresponds to fig. 4a, but in the example drawn the apertures are coalescent in both vessels. Fig. 5 (2) is similar to fig. 4c, but the walls of the contiguous vessels differ in thickness.

The vessels never touch the rays, but are always separated from them by at least one cell of paratracheal parenchyma.† There are therefore no pits from vessels to ray cells, but only from vessels to wood parenchyma. In all the genera of the Sterculiaceae except *Brachychiton*, *Firmiana* and *Sterculia* the vessel-parenchyma pit-pairs are similar

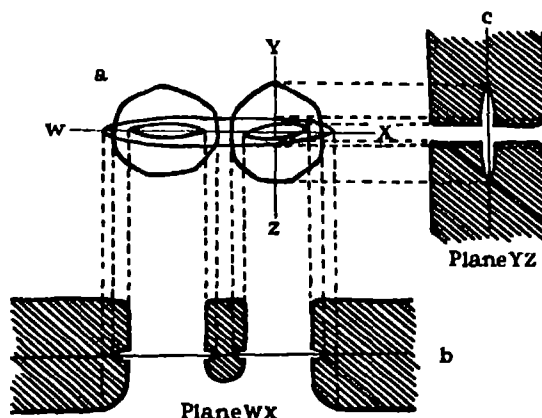


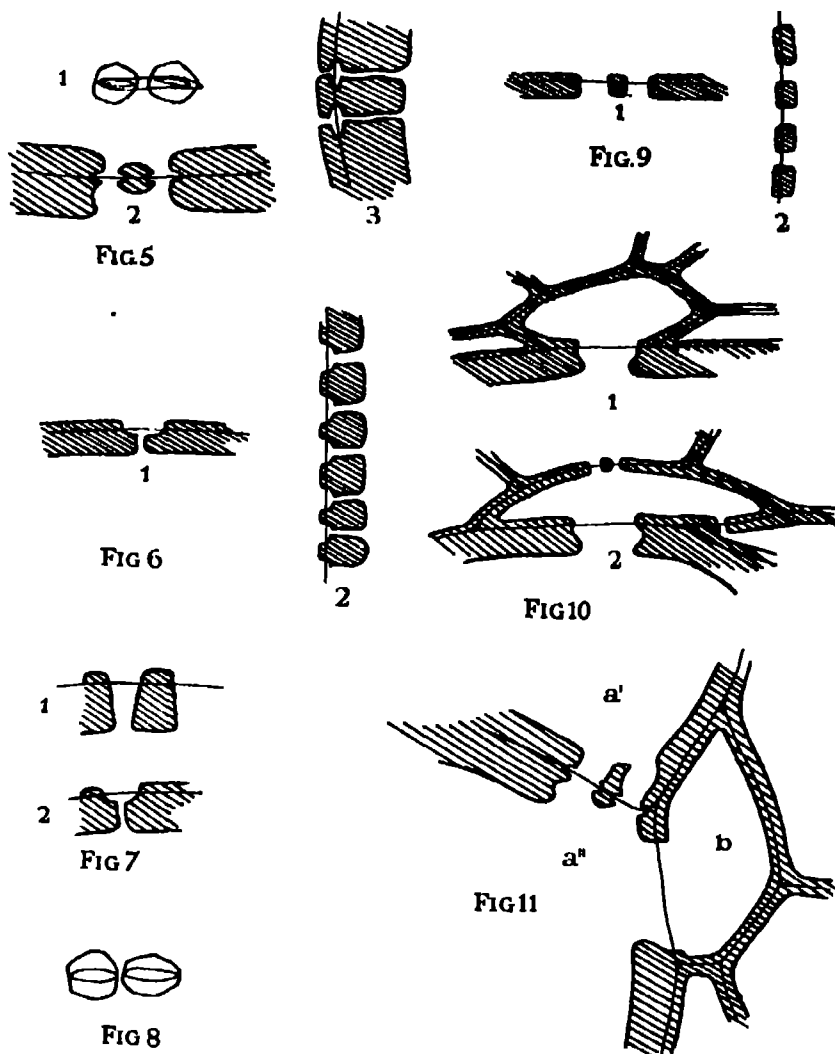
FIG. 4—Diagram of two intervascular pit-pairs, with coalescent apertures in one wall.
a, tangential section; b, cross-section; c, radial section.

in size and shape to the intervascular pit-pairs, but consist of one simple pit in the parenchyma, and one bordered one in the vessel. Such a pit-pair is shown diagrammatically in fig. 12. The surface view is very similar to that of the intervascular pit-pairs, but the sectional elevations show a bordered pit on one side only of the middle lamella; the other pit, in the parenchyma cell, is simple. A pit-pair of this type, from the wood of *Sterculia quinqueloba* K. SCHUM., is illustrated in fig. 6.

In all the bordered pits so far described the secondary wall arches over the primary pit field, producing a pit in which there is a sudden narrowing of the pit chamber towards the outer aperture. If the apertures of two or more pits coalesce, this inner aperture is very large, as in figs. 4 and 5. The pit canal, however, is not always of equal width throughout its length, and though it most commonly widens towards the inner aperture, it may occasionally narrow or curve. This is possibly due to the later deposits of the secondary wall being laid on in such a way as to arch over the earlier

* Coalescent apertures: slit-like inner apertures united into spiral grooves.

† Paratracheal parenchyma: see footnote, p. 331.



FIGS. 5-11—Intervascular and vessel-parenchyma pit-pairs. (All approx. $\times 650$.)

FIG. 5—*Sterculia quinqueloba* K. SCHUM. Intervascular pit-pairs. 1, surface view; 2, cross-section; 3, longitudinal section.

FIG. 6—*S. quinqueloba* K. SCHUM. Vessel-parenchyma pit-pairs. 1, cross-section; 2, longitudinal section.

FIG. 7—*S. urens* ROXB. Vessel-parenchyma pit-pair. 1, simple pit in vessel wall; 2, bordered pit in vessel wall; 1 and 2, both in cross-section.

FIG. 8—*S. quinqueloba* K. SCHUM. Intervascular pit-pair, with wide aperture extending as far as the outline.

FIG. 9—*S. ornata* WALL. Simple vessel-parenchyma pit-pairs. 1, cross-section; 2, longitudinal section.

FIG. 10—*S. quinqueloba* K. SCHUM. Simple vessel-parenchyma pit-pairs. The vessel wall arches over the primary pit field, but no distinct pit chamber is formed. 1 and 2, cross-section.

FIG. 11—*S. quinqueloba* K. SCHUM. Group of vessel-parenchyma pit-pairs in cross-section. *a'* and *a''* are vessels, and *b* is a parenchyma cell; the pits from *a''* to *a'* are bordered, the pit from *a''* to *b* is simple.

ones. This overarching of the later deposits of the secondary wall is clearly shown in fig. 5 (2). It may also occur when the pits are simple, and it is easy to confuse the resulting simple pit with one that has a true border. The pit-pairs of *Sterculia ornata* WALL. illustrated in fig. 9 are simple, and so are those of *Sterculia quinqueloba* K. SCHUM. shown in fig. 10. These pit-pairs become more understandable when they are examined at a high magnification; seen with a low-power lens they appear bordered, but with a higher magnification it becomes clear that there is no distinct pit chamber, and that the apparent border is really an overarching secondary wall. A simple and a bordered pit from *Sterculia urens* ROXB. is illustrated in fig. 7; in each case the pit leads to a parenchyma cell, and the distinction between the two types is clear.

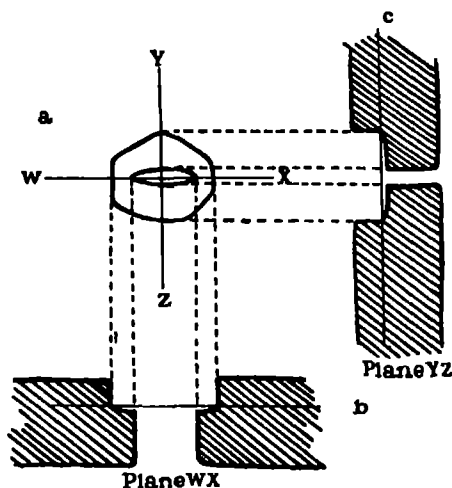


FIG. 12—Diagram of vessel-parenchyma pit-pair. *a*, tangential section; *b*, cross-section; *c*, radial section.

In *Firmiana* the majority of the vessel-parenchyma pit-pairs are similar to those described above, but unilaterally compound* pits also occur. A single oblong pit in the parenchyma cell may subtend several pits in the vessel wall (fig. 3). This type of pit is very common in *Firmiana*, and occurs occasionally in species of *Sterculia* A and in *Brachychiton*. In the last two genera there are both unilaterally compound pits and vessel-parenchyma pit-pairs that are much larger than the intervacular pit-pairs; these are illustrated in fig. 3; they are characteristic of all the species of *Sterculia* A of *S. quinqueloba* and of all the species of *Brachychiton*.

An interesting group of pit-pairs was seen in cross-section in *S. quinqueloba*; it is illustrated in fig. 11. The section passes through the junction of three cells, *a'* and *a''* are vessels, and *b* is a parenchyma cell. The pit-pairs between the vessels are bordered, and one of the pits in *a''* has an overarching wall as well as a marked border. The pit-pair between the vessel *a''* and the parenchyma cell is simple, though there is again some overarching of the vessel wall. The apertures of all three pits in the vessel *a''* are

* *Unilaterally compound pitting*: a type of pitting in which one pit subtends two or more smaller pits in the cell adjacent.

coalescent. In *S. quinqueloba*, although a great number of apparently simple pits from vessels to parenchyma cells are seen on cross-sections of the wood, fewer are to be found on longitudinal sections. The explanation of this appears to be that even when the pits from vessels to parenchyma cells are the same size as the pits to other vessels, the apertures are often much larger than those in the intervacular pit-pairs, especially horizontally. In extreme cases the aperture extends as far as the outline, with the result that the pit may appear simple when cut through transversely, although when it is cut longitudinally a large border can be seen. Fig. 13 shows a surface view and sectional elevations of two such pits. In *b* both pits appear simple, but in *a* and *c* a border can be seen. Fig. 8 illustrates two such pits from *Sterculia quinqueloba*.

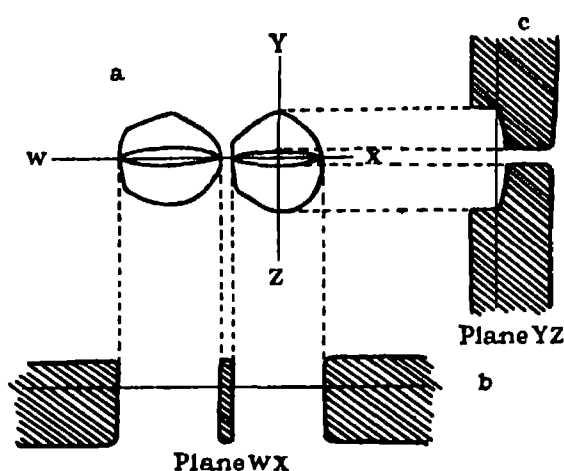


FIG. 13—Diagram of vessel-parenchyma pit-pair with wide aperture extending as far as the border. *a*, tangential section; *b*, cross-section; *c*, radial section.

In a study of large pit-pairs between vessels and parenchyma cells recently made at this Institute, Ross (1936) has attempted to estimate their significance. From his work, as well as from this investigation, one fact emerges clearly, that large vessel-parenchyma pit-pairs do not occur unless the intervacular pitting is large. Ross states that they only occur in "those woods whose intervacular pits are round, alternately arranged and have a diameter greater than about six microns". In the Sterculiaceae, too, they only occur where the pit-pairs are large, but even then they are not universal in all genera. From a superficial survey of their occurrence in other families the author has been led to the conclusion that they are usually developed with some regularity throughout a family (Lauraceae) or throughout certain sections of a family (Bombacaceae, Rosaceae), and that it is less common to find them, as here, in single genera and not in closely related genera.

RECORD (1934) states that in woods with storeyed structure the vessel-parenchyma pit-pairs are mostly small and half-bordered. *Sterculia* and *Brachychiton* are exceptions to this, and so are many genera of the Bombacaceae and some genera of the Tiliaceae. There are also a few exceptions in other families (one in the Amarantaceae, two in the

Caesalpiniaceae and one in the Ulmaceae), but only very few, and it may be significant that of the genera with large vessel-parenchyma pit-pairs and regular storeyed structure, by far the majority belong to families of the Malvales. The main tendency in the phylogeny of pitting has been for a reduction in the size of the pits, and FROST's (1931) series from scalariform to alternate is supported by correlation with the vessel-member length. Large vessel-parenchyma pit-pairs do not fit into any such series. They do not conform to any of the accepted types of pitting, and their frequent orientation with the long axis vertical or oblique suggests that their scalariform shape is of secondary origin, and not related to primitive scalariform pitting. ROSS (1936) reviewed this type of pitting in several different families, but was unable to suggest any very definite correlations with other features.

In the Sterculiaceae the pitting is moderately constant in size and shape throughout each genus, and the smaller intervacular pit-pairs occur on the whole in the genera with the shortest vessel members. In the *Sterculia* A group there are no exceptions, and in the *Sterculia* B group *Sterculia pallens* WALL. has unilaterally compound vessel-parenchyma pit-pairs similar to those of *Firmiana*. This species is also exceptional in other respects, and its systematic position will be discussed later.

The two genera with large vessel-parenchyma pit-pairs are *Brachychiton* and *Sterculia* A, and these two genera have also many other features in common. The two sections of *Sterculia* are quite distinct in regard to the size and number of the pits, except for *S. quinqueloba* K. SCHUM. and *S. coccinea* ROXB.; these species are also intermediate between the two sections of the genus in the distribution of the parenchyma, and must be considered borderline types.

VIII—FIBRES

The fibrous tissue in all the genera of the Sterculiaceae is consistently *libriform*, with simple pits, and, with the exception of the crystal-bearing fibres, never *septate*. The fibres differ considerably in length, in the regularity with which they are storeyed, and in wall thickness, both from genus to genus, and occasionally within the genus.

The fibre length for the different genera is shown in fig. 14, in which the genera are arranged with the fibre length decreasing from top to bottom of the list. If this figure is compared with the one for vessel-member length (fig. 1) it will be seen that the position of many of the genera is reversed; for example, *Cola* and *Sterculia* B have very long fibres but short vessel members, while *Pterocymbium* and *Scaphium* have short fibres and long vessel members. The difference between the two sections of *Sterculia*, however, is not significant, and the fibres of *Sterculia* A are actually very slightly longer than those of *Sterculia* B.

One interesting feature of the fibre length is the relation between the ultimate length of the fibres and the length of the cambial initials from which they were formed, as inferred from the vessel-member length. The author has recently shown (CHATTAWAY

1936) that among woods selected at random from a very wide range of families the shortest cambial initials are associated with the greatest amount of extension in the fibres; for example, in woods with initials 350μ long, the fibres tend to be three times that length, while in woods with cambial initials of 1000μ and over, the fibres are rarely more than one and a half times the length of the initials, consequently the same fibre length may occur in woods with different lengths of cambial initial, and it is possible that the amount of extension may serve to distinguish the fibres of two woods where the actual length will fail.

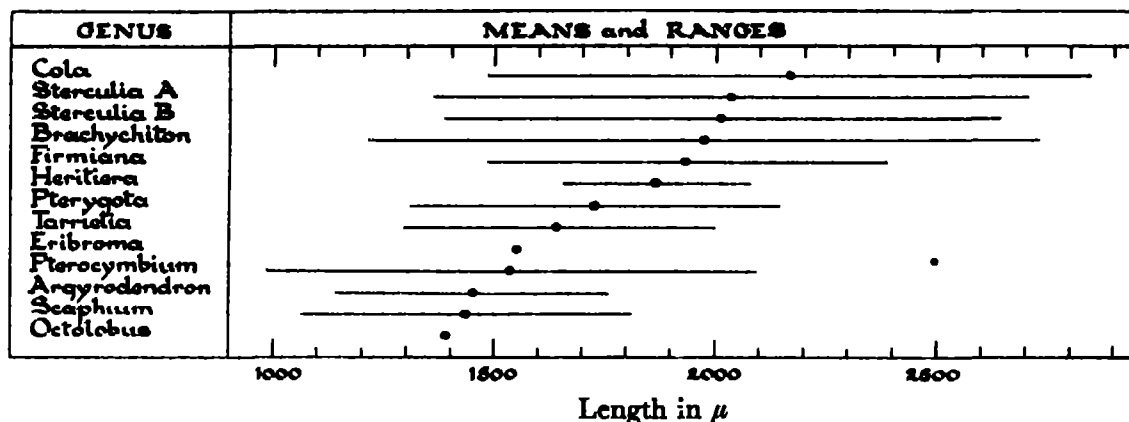


FIG. 14—Diagram to show fibre length in the Sterculiaceae.

The data given in figs. 1 and 14 for vessel-member length and fibre length have been combined in Table II to show the amount of fibre extension for different vessel-member lengths, and it will be seen that the amount of extension increases as the vessel-member length becomes shorter.

TABLE II—FIBRE LENGTH EXPRESSED IN TERMS OF VESSEL-MEMBER LENGTH

Genus	Fibre extension			Vessel-member length
	Fibre length			
	Vessel-member length			
	Mean of means	S.D. of mean		
<i>Pterocymbium</i>	2.88	±	0.44	Approx. 500 μ
<i>Scaphium</i>	2.93	±	0.56	
<i>Argyrodendron</i>	3.60	±	0.41	
<i>Tarratia</i>	3.95	±	0.56	400–500 μ
<i>Brachychiton</i>	4.24	±	0.58	
<i>Pterygota</i>	4.62	±	0.43	
<i>Sterculia A</i>	4.63	±	0.85	
<i>Firmiana</i>	4.81	±	1.01	
<i>Heritiera</i>	5.12	±	0.57	Below 400 μ
<i>Octolobus</i>	5.25			
<i>Cola</i>	5.70	±	0.71	
<i>Sterculia B</i>	5.73	±	0.91	
<i>Eribroma</i>	6.45			

Distinctions that can be drawn between the genera on the length of their vessel members are confirmed. In addition to this, although there is no significant difference

between the actual fibre lengths of *Sterculia* A and B, these two groups show a significant difference in the extension of their fibres.

The figures given in Table II were compared with those for fibre elongation generally (CHATTAWAY 1936); it was found that the values in the Sterculiaceae are consistently higher than those for a random selection of woods covering several families, though not higher than values met with in some other advanced families. Furthermore, they agree well with values calculated for this family from the data given by BAILEY (1920).

Unfortunately very little appears to be known about the causes of fibre extension, and consequently it is impossible to offer any explanation of the above phenomenon. But it is interesting to notice that in a recent discussion on libriform fibres and fibre tracheids (BAILEY 1936; REINDERS 1935), BAILEY comments on the fact that tracheids elongate less during differentiation than either fibre tracheids or libriform fibres. Many of the lowest values for elongation given by the present author (CHATTAWAY 1936) occur in woods with fibre tracheids, while high values predominate in families such as this, in which the fibres are libriform. This confirms the idea that there may be some phylogenetic significance to be attached to the type of pitting of the fibre walls, in spite of the overlapping of types that occurs in many families.

The fibres in some genera (*Cola*, *Sterculia* B, *Heritiera*, *Eribroma* and *Octolobus*) may show little sign of being storeyed, even where the parenchyma is regularly storeyed, the individual fibres being thin and thread-like, with little or no indication of a "bauchig" middle portion. These fibres show a greater amount of extension than the fibres of other genera that are "bauchig" and retain their storeyed arrangement. This suggests that the extreme amount of extension is only attained when the whole cell is involved. There are noticeably fewer pits in these very long fibres, which may be the result of the great extension they have undergone; in the regularly storeyed fibres the pits are almost all on the wider middle portion, and not on the attenuated tips, and they are often quite numerous; in the very long and thread-like fibres that are not storeyed the few pits that occur are not localized in any particular part of the fibres, but are scattered throughout their length.

Crystal-bearing fibres, which will be described in the section on crystalliferous cells, occur in *Sterculia* sp. and in *Eribroma*.

IX—VERTICAL WOOD PARENCHYMA

The three main types of parenchyma, terminal, metatracheal and paratracheal,* are all represented in the Sterculiaceae; the terminal parenchyma occurs sporadically

* *Terminal parenchyma*: aggregated wood parenchyma, forming a more or less continuous layer of variable width at the close of the season's growth. *Metatracheal parenchyma*: aggregated wood parenchyma forming concentric laminæ, mostly independent of the vessels and vascular tracheids. *Paratracheal parenchyma*: aggregated wood parenchyma in association with the vessels or vascular tracheids.

in many of the genera, and both metatracheal and paratracheal parenchyma occur together in all the genera except *Scaphium*.

Terminal parenchyma offers little that is of interest in this family; where it is well developed it forms lines on the cross-section, that are often continuous, and if the growing periods are short, these terminal bands may follow one another closely, and may be difficult to distinguish from broad bands of metatracheal parenchyma, as for example in some species of *Sterculia* and in *Argyrodendron*. Terminal parenchyma often occurs sporadically, and may be present in one sample of a species and absent from another.

The distribution of the metatracheal and paratracheal parenchyma shows considerable variation in different genera. Certain types can be distinguished which are linked together by intermediate forms. For example, the metatracheal parenchyma varies in distribution from narrow discontinuous lines one cell wide (the "diffuse"* type of the International Association of Wood Anatomists) to broad bands several cells wide, and there appears to be a sequence of development in these types that runs parallel to that for vessel-member length (CHATTAWAY 1932). The narrow lines occur in the genera with the long vessel members, and the broad bands in those with the shorter ones; and this sequence is accompanied by a parallel sequence in the type of cell of which the strands are composed. The cells of the narrow lines are often two per strand, and are then quite easy to distinguish from those of the paratracheal parenchyma (RECORD 1934); the cells of the broad bands are usually four per strand, and the individual cells of the metatracheal and paratracheal parenchyma are often quite indistinguishable from one another, and it is sometimes difficult to decide whether the bands are truly metatracheal, or are aliform and confluent, and consequently wholly paratracheal.

Pterocymbium javanicum R.BR. or *P. tinctorium* K. SCHUM. may be taken as an example of one end of the series. In these woods the fibres are very thin-walled and have large lumina, so that it is often difficult to distinguish between the fibres and parenchyma on the cross-section (fig. 25, Plate 29). It is, however, usually possible to distinguish the sheath of paratracheal parenchyma from the short tangential lines of metatracheal parenchyma. On tangential section the storeyed structure of the wood is clearly marked, the pointed ends of the parenchymatous elements interlocking with characteristic gabled ends (BEIJER 1927; RECORD 1934). The cells of the parenchyma are easy to recognize on the longitudinal sections (fig. 28, Plate 29) on account of their cross-walls, and the sheaths of paratracheal parenchyma are seen to consist of from four to eight cells (usually four), while those of the metatracheal parenchyma are usually subdivided into only two cells. Other strands can be seen that are clearly not fibres; they are of the same length as the parenchyma strands, and have similar gabled ends, but are not subdivided by cross-walls. These are the intermediate or substitute fibres of the

* *Diffuse parenchyma*: single parenchyma strands or cells distributed irregularly among the fibrous elements of the wood as seen on cross-section.

older writers (DE BARY 1884; SOLEREDER 1908), now called fusiform wood parenchyma cells,* as, in spite of their shape and their lack of cross-walls, they are certainly parenchymatous and not fibrous in nature. They may possibly represent a more primitive condition than the strands of two cells, before septation of the strands occurred at all. They are present in considerable numbers in some species of *Pterocymbium*, but the more usual condition throughout the genus is for the strands of metatracheal parenchyma to be formed of two cells, gabled at their extremities, and separated from one another by a cross-wall that meets the side walls at right angles, without any intercellular spaces. Three species of *Sterculia*, *S. crassiramea* MERRILL, *S. villosa* ROXB. and *S. cariboea* R.BR., are very similar to *Pterocymbium*, but as the fibre walls are slightly thicker in the two last-named species, it is easier to distinguish the tissues on cross-sections of these woods.

Cola (fig. 27, Plate 29) may be taken as representing the other end of the sequence. Here the broad bands of parenchyma are so conspicuous as to be visible to the naked eye on a cross-section of the wood. These bands, which are sometimes broad enough to include many of the vessels and their surrounding paratracheal parenchyma, are sharply marked off from the fibres, which form a solid mass, usually without any scattered parenchyma strands among them. The bands are composed of strands that are usually subdivided into four cells; the cells at the extremities of the strands have gabled ends that interlock with the strands of the storeys above and below; the middle cells of the strands have rounded corners and conspicuous intercellular spaces between them (fig. 30, Plate 29). There is no appreciable difference between the cells surrounding the vessels and those of the rest of the band, but the cells actually contiguous to the vessels are sometimes disjunctive,† and occasional strands on the edges of the bands may be formed of two cells instead of the usual four.

Intermediate forms exist between the two extreme types that have been described. The following genera have conspicuous lines of metatracheal parenchyma: *Brachychiton*, *Heritiera*, *Sterculia* A, *Tarrietia*; in these the fibres and parenchyma are more distinct on the cross-section than in *Pterocymbium*, and strands of four cells are of more frequent occurrence (figs. 26, 29, Plate 29). *Argyrodendron*, *Eribroma*, *Firmiana*, *Octolobus* and *Sterculia* B are very similar to *Cola*. *Firmiana* and *Pterygota* are usually very similar to *Cola*, but the bands sometimes appear as if they were wholly paratracheal. Thus, although in one part of a section the bands may be very regular, and appear to be formed independently of the vessels, merely including them where they interrupt the course of the bands, there may be other parts of the wood, even on the same section, where the direction of the bands seems to be influenced mainly by the position of the vessels.

* See footnote, p. 317.

† *Disjunctive parenchyma cells*: wood or ray parenchyma cells partially disjoined during the process of differentiation; contact is maintained by means of tubular processes. To replace "conjugate" parenchyma, which implies development in the wrong direction.

In a few species of *Sterculia* B, in which broad bands predominate (*S. coccinea* ROXB.; *S. quinqueloba* K. SCHUM.; *S. urens* ROXB.), there is also a considerable amount of scattered metatracheal parenchyma, and the general appearance of the wood is intermediate between the two types.

The strands that form the narrow lines of parenchyma are often less subdivided than those of the broad bands, and the cells fit tightly together and lack the intercellular spaces that occur between the cells in the broad bands. The position of the different genera in respect of these details is shown in Table III, from which *Scaphium* is omitted, as it has no metatracheal parenchyma.

TABLE III—THE VERTICAL WOOD PARENCHYMA

Genus	Metatracheal parenchyma predominantly in		Intercellular spaces	Number of cells per strand
	Narrow lines	Broad bands		
<i>Pterocymbium</i>	+	—	—	1-2
<i>Brachychiton</i>	+	—	—	2-(4)
<i>Sterculia</i> A	+	—	—	2-(4)
<i>Firmiana</i>	—	+	+	4
<i>Tarrietia</i>	+	—	—	2-4
<i>Argyrodendron</i>	—	+	(+)	(2)-4
<i>Cola</i>	—	+	+	4
<i>Pterygota</i>	—	+	(+)	4
<i>Sterculia</i> B	—	+	+	4
<i>Heritiera</i>	+	—	—	2-4
<i>Octolobus</i>	—	+	+	4
<i>Eribroma</i>	—	+	+	4

Brackets indicate that the feature or number occurs, but is uncommon.

In this table the genera are arranged according to vessel-member length, *Pterocymbium* having the longest, and *Eribroma* the shortest. It will be seen that broad bands, intercellular spaces and strands of four cells are on the whole characteristic of the genera with short-vessel members, while narrow lines, the absence of intercellular spaces and two cells per strand are characteristic of the genera with longer vessel members. Making use of FROST's (1930a, b) conclusions that the woods with shorter vessel members are the more advanced, the sequence given above suggests that in this family broad bands of parenchyma are a sign of specialization, and that this is accompanied by greater subdivision of the parenchyma strands and cells with rounded corners.

Two genera occupy anomalous positions as regards their parenchyma. *Scaphium* has relatively long vessel members and no metatracheal parenchyma, and is unlike the other genera of the Sterculiaceae in regard to this tissue, as it has narrow sheaths of vasicentric parenchyma* in most species, and only a little aliform parenchyma* in one

* *Vasicentric parenchyma*: paratracheal parenchyma forming a vascular sheath of variable width, and circular or oval in cross-section. *Aliform parenchyma*: vasicentric parenchyma with wing-like extensions. *Confluent parenchyma*: coalesced aliform parenchyma, forming irregular tangential or diagonal bands.

species, *S. wallichii* R.Br. *Heritiera*, on the other hand, has very short vessel members, but has nevertheless very abundant metatracheal parenchyma, in lines one cell wide. As will be shown later there are advanced features in other tissues in this genus, and advance in one tissue may have been accompanied by a lag in another.

Paratracheal parenchyma is considered by some writers to be a more advanced condition than metatracheal parenchyma, and EAMES and MACDANIELS (1925, p. 68) sum up the situation as follows: "In highly specialized woods, where the tissue consists largely of fibers, and where water-conducting cells—in such cases porous vessels—are relatively few, each vessel is ensheathed with parenchymatous cells, and no parenchyma cells occur among the non-conducting fibers." JEFFREY (1922, p. 59) also suggests that the gradual loss of conducting functions in the fibres, through the sequence tracheids—fibre-tracheids—libriform fibres, is accompanied by a gradual change in parenchyma arrangement, the concentration of parenchyma around the vessels replacing cells scattered among the fibres.

This idea of the relation between the parenchyma and the conducting tissues suggests that the development of homogeneous masses of parenchyma in connexion with the vessels is the most advanced type. As conduction has become localized in the vessels, contact of the parenchyma with the main conducting system has been retained by the development of the scattered cells into continuous bands, and the tendency appears to have been for these bands to have become wider and to enclose the vessels completely. But definitely paratracheal parenchyma, without any diffuse parenchyma, can be found associated with primitive vessel characters in many families (CHALK 1935), though in such cases it is never very abundant. Further specialization seems to have led to the development of more abundant paratracheal parenchyma, such as aliform and confluent; for example, in the Leguminosae the parenchyma is mainly vasicentric in the Mimosaceae, which is generally regarded as the least advanced subfamily, and mainly confluent in the Papilionaceae, which can be regarded as the most advanced. The study of parenchyma in the Sterculiaceae suggests that the greatest specialization is reached in the woods with broad bands that enclose the vessels, and that these have been derived partly from metatracheal and partly from paratracheal parenchyma.

Crystalliferous parenchyma strands occur in some of the advanced species of *Sterculia*, in *Eribroma*, *Argyrodendron*, and in some species of *Heritiera* and *Tarrietia*. They will be discussed in a later section.

X—RAYS

In two earlier papers on the Sterculiaceae (CHATTAWAY 1933*a, b*) the author published the results of investigations upon the rays of all the genera included in the family before its revision by EDLIN (1935). Two features were specially studied, the

cells* and sheath cells.† At that time the Sterculiaceae included several genera with tile cells, but since the revision of the family by EDLIN, these genera have been transferred to the Buettneriaceae, and therefore do not come within the scope of this paper. Tile cells (CHATTAWAY 1934; MOLL and JANSSONIUS 1906) occur in the nearly related families Bombacaceae, Malvaceae and Tiliaceae, but have not yet been observed outside this group. Sheath cells (MOLL and JANSSONIUS 1906) are rather more widely distributed, though by no means common, and occur in several other families. They are absent from the Buettneriaceae, but are present in the rays of all the genera of the Sterculiaceae except *Heritiera*. As a whole, with this exception, the rays of all the genera are markedly heterogeneous, having not only sheath cells, but also marginal cells, or marginal rows of cells, that are different in size and shape from the rest of the ray. As seen on the radial section both marginal cells, sheath cells and the cells of the uniseriate rays are upright, while the central cells of the multiseriate parts are procumbent. The rays thus conform to the type described by KRIBS (1935) as "heterogeneous II". The erect cells are usually without contents, but the procumbent cells in *Sterculia* sp., *Tarrietia*, *Argyrodendron* and *Heritiera*, are often full of dark contents, and in the other genera, although they are apparently without contents in the mature heartwood, there is often a distinction between the two types of cell in the sapwood, and the procumbent cells are often filled with starch, while the marginal cells appear quite empty.

In the evolutionary sequence of ray types that is given by KRIBS, this type of heterogeneous ray is less advanced than the homogeneous ray, uniformity of cell size and shape throughout the ray representing the highest form of ray development. *Heritiera* is interesting in this connexion. The ray type is more advanced than that of any other genus in the family; sheath cells are almost entirely absent, and the erect marginal cells that are so characteristic of the other genera are confined to relatively few rays, the majority of the rays having only a very slight difference between the marginal and central cells. In the other genera the uniseriate rays are formed of cells that are similar to the marginal and sheath cells of the multiseriate rays, thus conforming to KRIBS's type "heterogeneous II", while in *Heritiera* the cells of the uniseriate rays are usually small, and are similar to the procumbent cells of the multiseriate rays, thus conforming to KRIBS's (1935) definition of "homogeneous II". The marginal cells in *Heritiera*, if they differ from the central cells, do so in their radial dimensions rather than their height, so that they are often indistinguishable on the tangential section, though on the radial section they appear to be narrower radially than the central cells. There is, however, some variation throughout the genus, an heterogeneous rays are more common in *H. fomes* SYME., while homogeneous rays predominate in

* *Tile cells*: special type of apparently empty upright or square ray cells of approximately the same height as the procumbent cells and occurring in indeterminate horizontal series usually interspersed among the procumbent cells.

† *Sheath cells*: upright ray cells tending to form a sheath about the smaller cells of a multiseriate ray or the multiseriate part of a ray.

H. littoralis DRY. This specialization of the rays is confirmed by the vessel-member length, which points to *Heritiera* being a rather advanced type, although the parenchyma is less advanced, and consists of metatracheal lines one cell wide, and the paratracheal parenchyma is often very scanty.

Although the rays of the Sterculiaceae appear to offer little that is of interest in regard to the phylogeny of the family, there are two features of their development that are of general interest and have been followed in some detail. These are the method of increasing the number of rays as the girth of the stem increases, and the rather unusual method of growth of the individual ray initials that gives rise to sheath cells.

As the stem increases in girth there is a proportional increase in the amount of ray tissue, but this is not merely by the growth of existing rays which must become farther and farther apart, but by the formation of new rays to maintain the even distribution of the ray tissue. It has already been shown by BEIJER (1927) and others that new uniseriate rays arise by subdivision of the fusiform initials, which normally give rise to vertical wood elements, and this process is common in the Sterculiaceae; but in this family the spread of the ray tissue is also achieved by branching of the large rays. This breaking up of a large ray into smaller ones is brought about by the change of ray initials into fusiform ones. These two methods of spreading the ray tissue involve contradictory processes, in one instance the conversion of fusiform initials to ray initials, in the other the reversion of ray initials to the fusiform condition.

The multiseriate rays that are found in an old stem are not all primary in origin; some of them are derived from the new uniseriate rays, that have subsequently grown considerably in size. This increase in the number of cells is usually the result of the division of the ray initials, but it has been found that this process is augmented in some of the Sterculiaceae by absorbing adjacent fusiform initials into the rays. These new ray initials are at first larger than those of the rest of the ray, and give rise to the sheath cells.

In order to understand the structure of the rays in the mature wood, it is necessary to see how they have arisen, and to be able to visualize them as ribbons of tissue set edgewise in the wood, extending from pith to cortex, or starting in the mature wood, and usually extending to the cortex, but occasionally fading away again in the wood. It is clear that to achieve such a picture of the rays it is not enough to study the cambium alone, as it is essential to trace the history of individual cambial initials throughout the development of a multiseriate ray from its inception. Sections of the cambium show any particular cell at one stage only, and are therefore inadequate. Special methods were used for following the course of the rays from the pith outwards. They have already been described in § III. These methods are only suitable for a study of tissues in which there is little extra-cambial growth, that is to say, in which the individual cells undergo little change in size or shape after their formation from the cambium, and consequently there are limits to their use in the study of vessels or fibres, or of woods without a rather regular arrangement of the tissues. They

are, however, entirely suitable for an investigation of ray cells, in which extra-cambial growth is slight, except in a radial direction, and in which the cells retain their shape, and change so slowly in relative position that the products of any particular ray initial can be traced on serial tangential sections for a long distance through the wood, while any change of shape in the cambial initial is immediately mirrored in the daughter cell. Furthermore, it is easy to distinguish between intra-cambial subdivisions that affect the initials, and extra-cambial ones that take place in the daughter cells. The former will be seen in all subsequent daughter cells, and will permanently affect the appearance of the ray, while the latter occur only in one cell, and the changes they cause will be seen only in one section.

(a) *Increase in the Number of Rays*

Examination of very young stems shows that the primary wood of the Sterculiaceae forms a completely closed ring round the stem, and the primary rays, though they may be high, do not extend for the full internode, and are seldom more than two or three cells wide. During the formation of the first few rows of secondary cells the primary rays become much wider, as the result of radial divisions of their initials, and in some species they may be extremely large at maturity.

As secondary growth proceeds and the stem increases in girth the primary rays become widely separated, and the need arises for additional ray tissue; this is provided for in two ways. The most usual method is by the formation of new ray initials from fusiform initials, so that a great many small uniseriate rays appear between the large ones. The formation of a new ray initial from a fusiform cambial initial has been described before (HABERLANDT 1914), but there appears to be very little record in literature of the development of the newly formed uniseriate ray into one of the different types of multiseriate ray. The first stage of ray growth is the transverse division of a fusiform initial, and the resultant uniseriate ray may at first closely resemble a parenchyma strand, but the cells soon become slightly more rounded. As the usual procedure throughout the family Sterculiaceae is for only one, or occasionally two or three superposed fusiform initials to be used up in the formation of the new ray, and as the fusiform initials are storeyed, it is common to find numerous short uniseriate rays arranged in regular horizontal rows. In many genera these uniseriate rays are very numerous, and they often form such a conspicuous feature of the tangential section of the wood as to give the impression of two distinct ray sizes, as if the uniseriate rays belonged to a different system from the multiseriate ones. In very young stems this is the case, and the rays are either primary and multiseriate, or secondary and uniseriate, but some of the uniseriate rays soon grow, and this condition is rapidly succeeded by one in which the multiseriate rays are both primary and secondary in origin, and represent all gradations in size from the biseriate condition upwards.

Although this method of spreading the ray tissue evenly through the stem is the

most usual it is not the only one. There are some woods in which uniseriate rays are only sparingly developed, and the spread of ray tissue occurs mainly by the breaking up of the larger rays into smaller ones, which continue to grow and then again divide. This splitting up of large rays has already been described by ZIJLISTRA (1909) and JOST (1901) for the primary rays of the Cupuliferae, for *Aristolochia* and *Clematis*, and for the secondary rays of *Fagus*, but the process has not been followed in great detail,

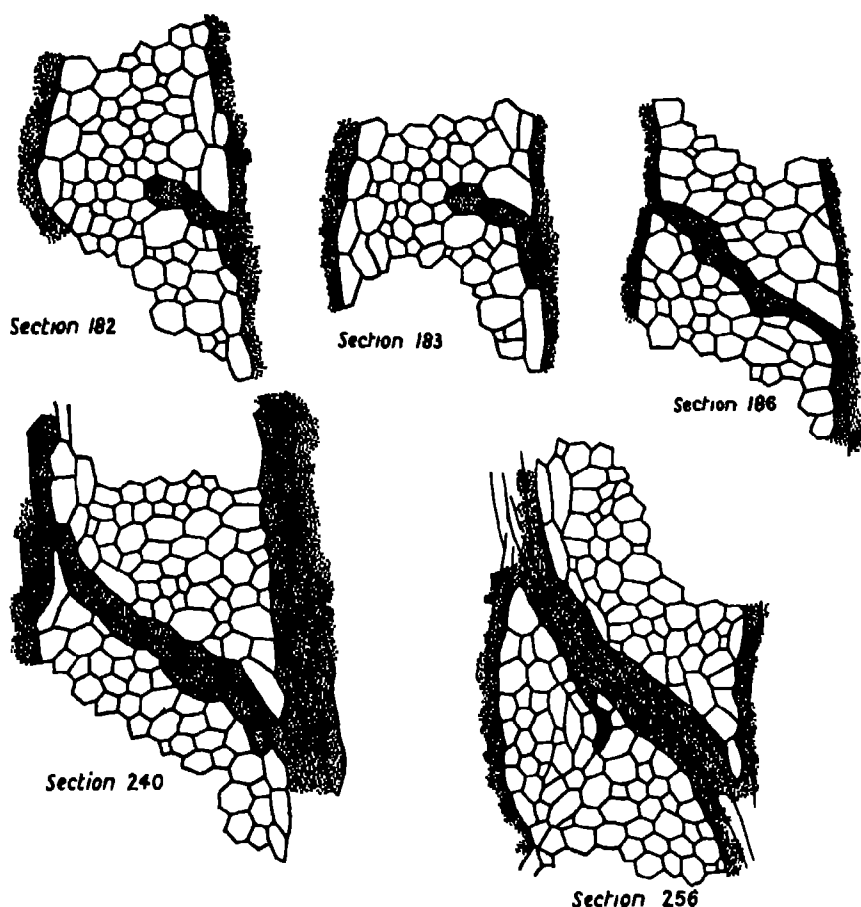


FIG. 15—*Cola togoensis* ENGL. and KRAUSE. Reversion of ray initials to fusiform initials, causing separation of a large ray into two parts. In section 240, the ray lies among wood parenchyma, in section 256, among fibres. ($\times 85$ approx.) Reproduced by permission from *Forestry*, 7 (2).

and no adequate explanation has been offered for its occurrence. Jost suggests that it occurs by the return of ray initials to the fusiform condition, but gives neither diagrams nor further explanation, and later refers to adjacent fusiform initials pushing in between the ray cells by sliding growth.

This process has been followed in different genera of the Sterculiaceae, especially in *Cola togoensis* ENGL. and KRAUSE. Details of the changes are given in fig. 15. The figure shows camera lucida drawings selected from serial sections 30μ thick passing

through approximately 2.2 mm. of wood at a distance of 5.5 mm. from the pith. In sections 182 and 183 from the pith the first stages are seen. They involve a slight swelling and elongation of the ray cells; they are made more conspicuous in the drawing by the cross-hatching, which does not imply a difference in the cell contents, but merely indicates the cells in which any change is recognizable. They might not be recognized as the preliminaries to a split if they were seen only on isolated sections, but can be recognized easily where the later stages can be traced backwards to their inception. In section 186, the line of slightly elongated cells is clearly seen across the ray; in section 240, the ray is passing through one of the layers of wood parenchyma, and is seen to be cut right across by a layer of cells that is almost identical with the parenchyma on the right; in section 256, the ray is in a region of fibres, and the separating layer is fibrous too. Thus it is clearly seen that initials that were producing ray cells are now forming alternating bands of fibres and vertical wood parenchyma; and this is the normal sequence for fusiform initials in this wood. It should be quite clear from this diagram that there is no question of adjacent initials pushing in between the ray cells, for the individual cells can be recognized in all sections, and they themselves are changed, not pushed aside by intruding cells.

Similar breaking up of the larger rays has been observed in species of *Cola*, *Sterculia*, *Eriobroma* and *Pterygota*. In *Cola togoensis* it occurs with extraordinary frequency, and appears to be the chief way of providing the amount of ray tissue necessitated by the increasing perimeter of the stem, and of disseminating it through the stem. New uniseriate rays which provide for this increase in other woods are infrequent. Fig. 16 shows a series of diagrams selected from a series of 1500 sections at intervals through approximately 8 cm. of stem, starting from the pith. The wood has moderately numerous primary rays that often extend some distance vertically, though not through the whole primary internode. Five such rays are shown in fig. 16 (1); they are marked by various signs and cross-hatchings to enable each to be followed in successive sections, and they are marked *A* to *E*. At the stage shown in (1), which is near the pith, the wood is not yet storeyed, but tangential growth is very rapid, and storeys are established within a few hundred sections of the centre of the stem. Fig. 16 (2) shows the first indication of any splitting, where the changes already described in fig. 17 occurred, and the rays *A*¹ and *A*² are seen to be cut off from *A*; by (5) these three rays are seen to be separated by a considerable mass of ground tissue. In (5) ray *B* begins to split, and similar splits can be followed in the other rays in succeeding sections. Ray *F* appears first as a small uniseriate ray in (3); it grows till (14), when it undergoes its first split into *F* and *F*¹. Ray *C* splits for the first time in (7); some of the rays derived from it are pushed out of the section by the tangential growth of the wood, and do not appear in the diagrams, but nevertheless by the end of the series ray *C* can be seen to have given rise to ten rays, and to have spread through a considerable tangential area of the wood.

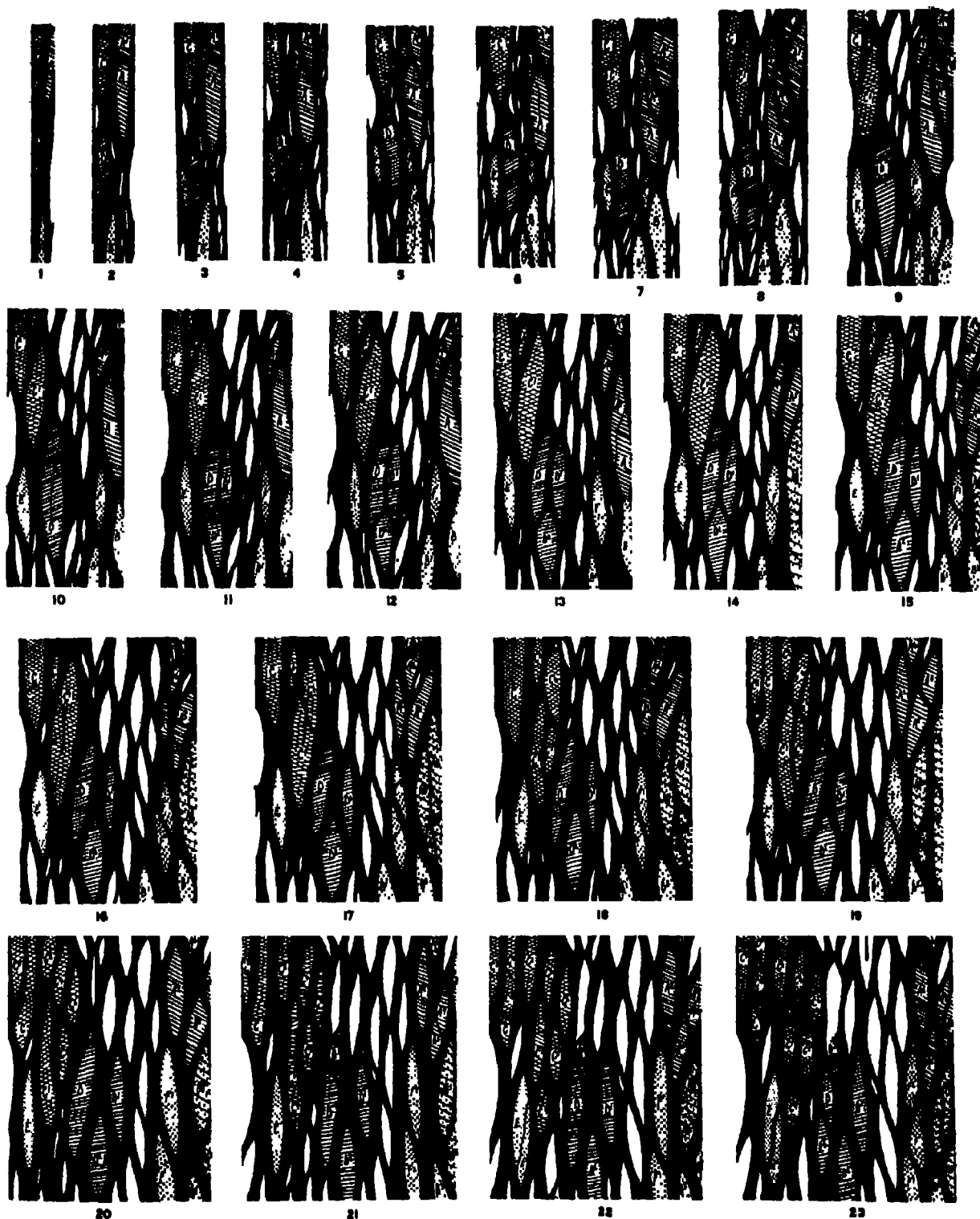


FIG. 16—*Cola togoensis* ENGL. and KRAUSE. Development of the rays through 8 cm. of wood, showing increase in number by splitting. No. 1 from a section near the pith. The cross-hatching of each ray shown in No. 1 is retained throughout the series for that ray and its derivatives. ($\times 5$.) Reproduced by permission from *Forestry*, 7 (2).

(b) Increase in the Size of Individual Rays

The two methods by which the rays are spread through the wood are thus seen to be by the formation of new uniseriate rays, and by the splitting up of large ones. Growth in size may also take place in two different ways. Rays usually increase the number of their cells by the swelling and division of the ray initials, the peripheral ones being larger and dividing more actively than those in the middle. This type of ray growth

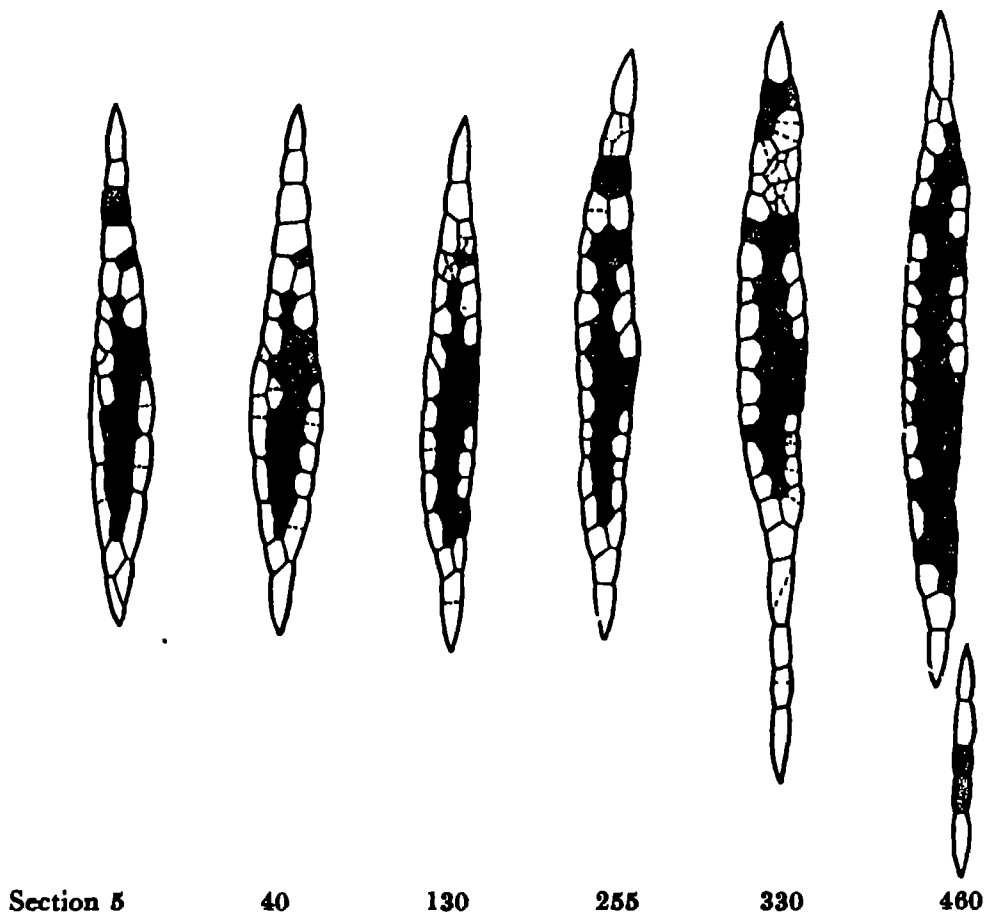


FIG. 17—*Tarrietia utilis* SPRAGUE. Gradual subdivision of added initials, forming sheath cells, and ultimately procumbent cells. The fusiform initial which is added to the bottom of the ray in section 330 is subsequently separated from it, and gives rise to an independent uniseriate ray (section 460). ($\times 85$.) Reproduced by permission from *Forestry*, 7 (2).

adds to the width of the ray, and at the same time to the circumference of the stem by the production of new cells. Rays may grow in height by the cells at the apex swelling, pushing up between adjacent fusiform initials, and then dividing. Occasionally growth in height may take place more suddenly by a fusiform initial above or below the growing ray changing into a series of ray initials. This is shown in fig. 17 for *Tarrietia utilis* SPRAGUE. The newly added initial usually remains attached to the existing ray, and grows with it, but occasionally, as in the ray figured, it may become

separated from the larger ray while it is still in the uniseriate condition, in which case it continues to grow as an independent ray.

This method of growth by converting adjacent fusiform initials to ray initials and their addition to an existing ray is the second method of growth already referred to. The addition of a whole fusiform initial to increase the height of a ray is of moderately common occurrence; less common is the addition of new initials to the sides of the rays, resulting in rays with sheath cells. These new initials are at first only subdivided into three or four cells, and are very similar to parenchyma strands, which may indeed easily be mistaken for newly added ray initials. Once they have been added to the ray,

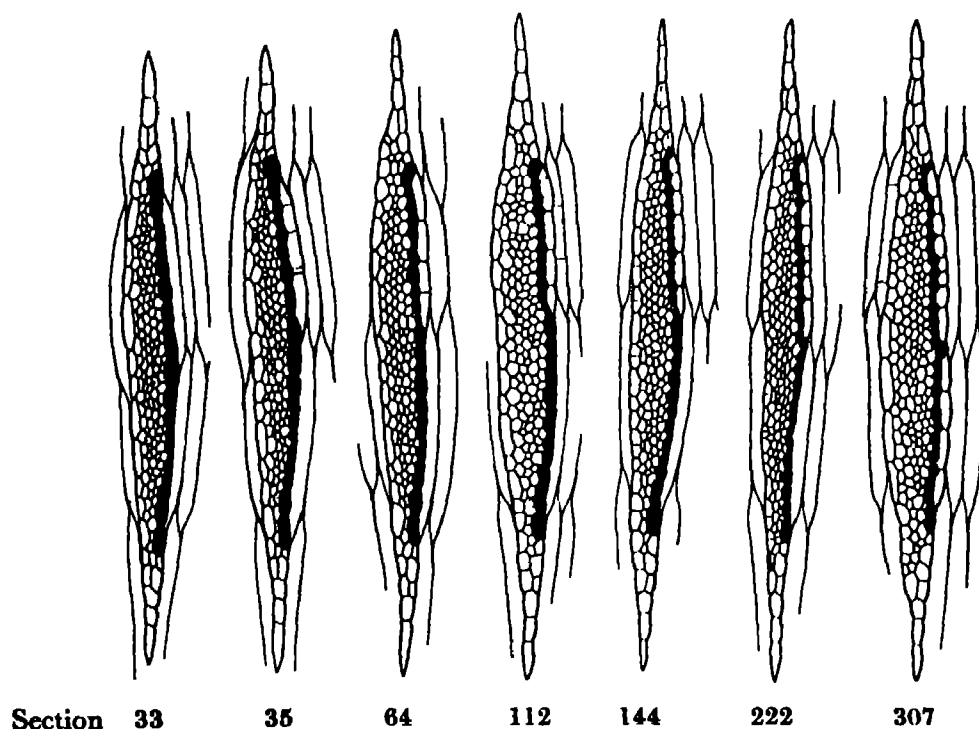


FIG. 18—*Pterocymbium javanicum* R.Br. Formation of sheath cells by the addition and subdivision of adjacent fusiform initials. (\times approx. 45.) Reproduced by permission from *Forestry*, 7 (2).

however, these initials persist, and appear in all subsequent sections, whereas a parenchyma strand, which results from extra-cambial subdivision of a daughter cell of an initial, is only transitory, and gives place in subsequent sections to the usual radial sequence of fibres and parenchyma.

It is this process of adding a layer of cells down the side of the ray that gives rise to the marginal sheath of larger cells by which the rays of this family are so often surrounded. Details of their formation have been studied in *Pterocymbium javanicum* R.Br., in which there is often a very conspicuous layer completely surrounding the rays. Fig. 18 shows a series of camera lucida drawings selected from serial sections 30μ thick through approximately 1.5 cm. of mature wood. In order to follow the

changes in size and position more easily all the peripheral cells on the right of the ray are cross-hatched in the first drawing, and the same cells are similarly marked in all the subsequent ones. These cells are larger than the central cells of the ray, and are bordered by successive fibrous or parenchymatous cells of the ground tissue of the wood. In section 35, the cell lying immediately to the right of the ray in the upper storey is subdivided, and looks like a parenchyma strand. This subdivision has, however, taken place in the initial, and not extra-cambially, and it persists through all subsequent sections. This new cell layer is now the peripheral layer of the ray, which has increased in width, and it replaces the former peripheral layer, the cells of which are undergoing further subdivisions, and becoming smaller. The newly added cells are at first large, but they become smaller through the formation of transverse walls, and ultimately assume much the same proportions as the cell row to which they were added. Meanwhile, similar changes have been taking place in the lower part of the ray. The shaded peripheral cells in the lower part of the ray have undergone subdivision, and in section 307 a new initial is added to the ray in the lower storey, and at the same time another initial is added to the opposite side of the ray. In section 222 the lower part of the ray appears much narrower than it was in section 144, which may possibly correspond with the change from erect to procumbent cells. It is not possible to check this fact, as it would necessitate being able to see both radial and tangential sections of the same cell, but the slight shrinkage in tangential area that is frequently seen in the initials at this stage of ray growth is probably associated with extra-cambial radial elongation of the ray cells. It is quite distinct from the swelling and shrinking in ray width that commonly occurs when a ray is passing through a parenchymatous or fibrous layer in the wood respectively. The decrease in width mentioned here occurs irrespective of the tissue which surrounds the ray at the time. It will be seen that in both section 144 and section 222 the cells adjacent to the ray are fibrous.

Fig. 19 shows a more general view of ray growth in the same wood. The sections from which the drawings in fig. 18 were made occurred between fig. 19 (3) and (4) in this figure. The whole range of diagrams in this figure represents sections at intervals of approximately 1.5 cm. through 9 cm. of wood. Fig. 19 (1) shows two rays, one three storeys high, and the other, only partly shown, thirteen storeys high. These large rays are separated from one another by one fibre, and each is surrounded by a conspicuous sheath of large cells. On their left are two uniseriate rays, each of which has arisen through the change of a fusiform initial into a series of ray initials, and subsequently grown by division of the cells till it exceeds its original storey in height. The growth of these rays can be traced through the whole 9 cm.; at the end of the series they are seen to be 4 and 5.5 storeys high respectively, and to have acquired a complete layer of sheath cells. The increase in size has taken place in two ways, by cell division and by the conversion of fusiform initials to ray initials, and their addition to the existing cell complex. Growth in height has been gradual, except in Fig. 19 (3),

by swelling and subsequent division of the apical initials, which push their way in among the surrounding elements. This accounts for the fact that increase in height is, for the most part, independent of the storeys. The exception to this is between Figs. 19 (2) and (3), where one of the small rays is seen to have jumped a complete storey by the addition of a whole converted fusiform initial.

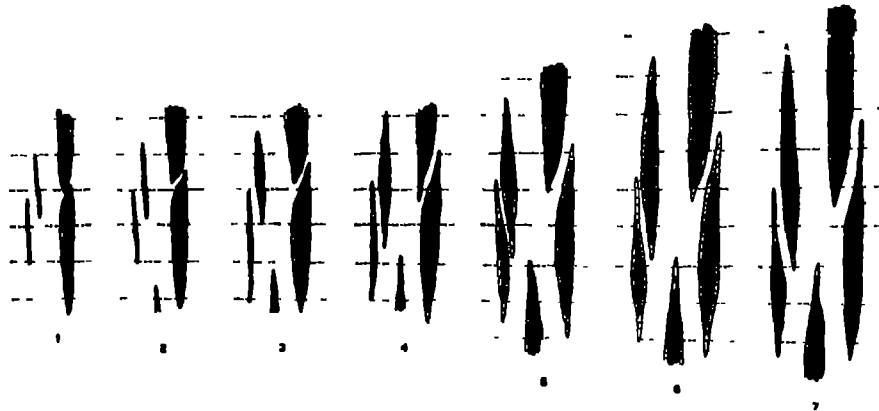


FIG. 10—*Pterocymbium javanicum* R.Br. Successive sections at 1.5 cm. intervals, showing the development of two uniseriate rays. The dotted horizontal lines represent the limits of the storeys in the other elements. ($\times 10$.) Reproduced by permission from *Forestry*, 7 (2).

Figs. 17 and 20 show similar stages in the addition of a layer of sheath cells to a ray of *Tarrietia utilis* SPRAGUE. The distinction between the marginal and sheath cells is very noticeable in this species, as the procumbent cells of the ray are usually filled with dark contents. In figs. 17 and 20 the cross-hatching represents the dark contents of the cells, and it is clear that the contents appear in the peripheral cells after the large sheath cells have been subdivided, and this is probably also the time at which the cells elongate radially and become procumbent. The author showed (CHATTAWAY 1933a) that in rays with tile cells there is probably some connexion between the elongation of the procumbent cells and their dense contents, and that lack of contents and a different appearance in the cell contents of the initials is associated with the erect tile cells. It has not been possible to examine fresh cambial material of *Tarrietia*, but the sudden change from empty cells to cells filled with dense contents in the mature wood may possibly reflect some fundamental difference in the initials from which the cells have been derived.

These sheath cells, which are so conspicuously developed in *Pterocymbium* and *Tarrietia*, are present in all the genera except *Heritiera*. In some genera they are much less regular than in others, notably in *Cola*, *Sterculia* B, *Eriobroma* and *Octolobus*, and often form only a partial sheath around the ray. In this case growth takes place mainly by division of the existing initials, supplemented by the occasional addition and conversion of fusiform initials.

This method of growth is evidently at the expense of the fusiform initials that form the ground tissue of the stem, and it contributes nothing to the increase in perimeter.

In rare cases—as, for example, in ray D^3 in fig. 16 (22, 23)—it may use up all the fusiform initials between the rays, so that they fuse into one. But this is unusual, because the fusiform initials generally multiply by radial division more rapidly than they are absorbed by the rays.

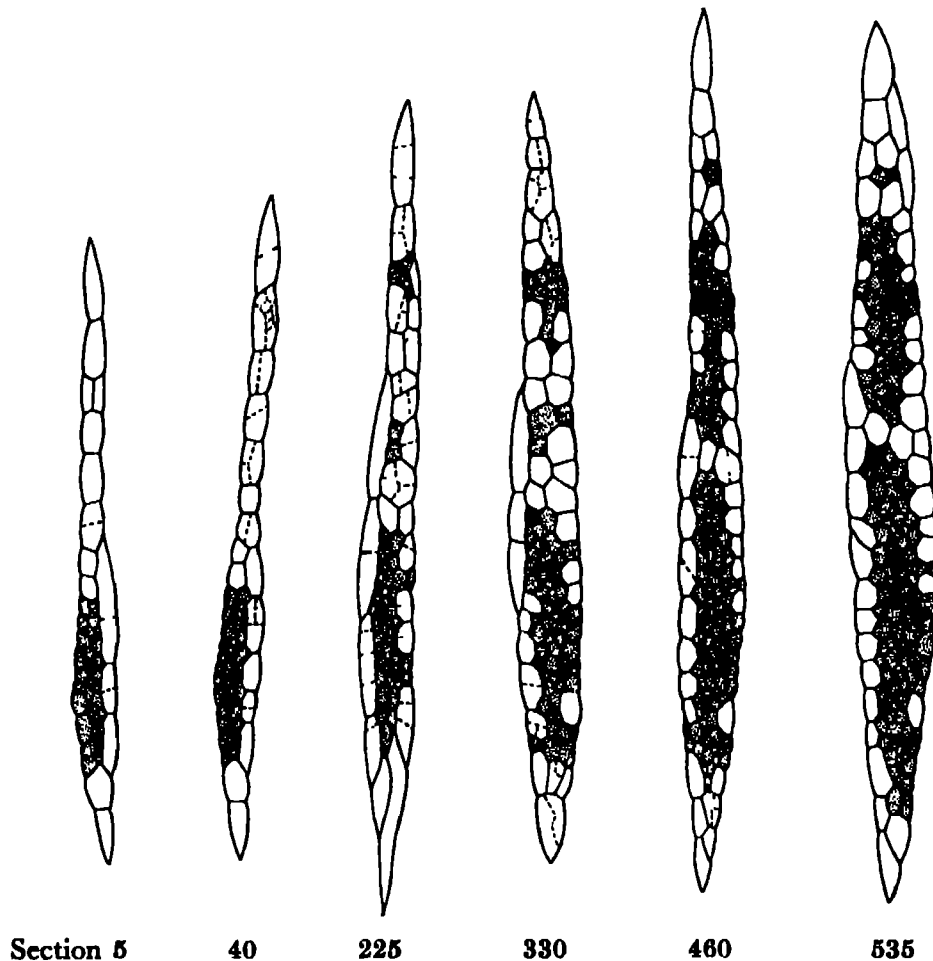


FIG. 20—*Tarrietia utilis* SPRAGUE. Gradual subdivision of added initials, forming sheath cells, and ultimately procumbent cells. The shading represents the cell contents that appear in the peripheral cells after the large sheath cells have been subdivided. ($\times 60$.)

The phenomenon of splitting rays in this family appears to have little systematic importance, and to depend almost directly upon ray size. The concentration of growth into a few very large rays has two results: first, it produces a very uneven distribution of ray tissue through the stem, large patches of ray tissue and large spaces without any, and, secondly, it produces rays in which the peripheral cells that are in contact with other elements of the wood are few compared with the number of inner cells that are surrounded only by other ray cells. Thus the splitting up of a ray has two effects, the ray tissue is spread more evenly through the stem, and the surface of the rays is increased in proportion to the bulk. A study has been made of the ratio of ray surface to mass in

the Sterculiaceae, using the perimeter and the area of the ray as seen on the tangential section, but it is impossible to say more than that it confirms the view that the splitting process is closely related to a low ratio. In the species with smaller rays, and in the small rays of all species, this ratio is very high; with slight increase in the size of the rays it falls very rapidly, since the area is approximately doubled when a uniseriate ray becomes biseriate, while the increase in surface is small. In the larger rays the ratio decreases more slowly, and the result is a curve that is steep at first, but finally flattens out, a common form of growth curve. For each species there seems to be a "danger zone" on this curve, and when this ratio is reached the ray is liable to divide.

In *Sterculia quinqueloba* K. SCHUM., although the rays were traced through nearly 3000 sections—representing approximately 9 cm. of wood—no complete splitting was observed. Several times a ray began to split, and several ray initials reverted to the fusiform condition, but the split was never completed, and the ray either remained in this condition permanently, or, more frequently, the reverse process took place and the split closed up. During this time the ray did not grow in size. Similarly in *Sterculia urens* Roxb., rays began to split, often in considerable numbers; these rays were followed in sections through about 15 cm. of the wood, and the initials that had changed always reverted to fusiform initials. Finally, however, some factor must have intervened to upset the balance, for within a few millimetres several rays were found to have completed the split. In both woods it was very noticeable that the splits almost always started from the same end and side of the different rays, which suggests a physiological impulse passing up or down the tree. It has not been possible yet to procure material in which the top could be distinguished from the bottom of the specimen, so this point remains unsettled.

Reviewing this family as a whole, it appears that the more primitive method of ray growth is by the addition of new fusiform initials, since the woods with the longest vessel members have the more regular layers of sheath cells (*Pterocymbium*, *Brachychiton*, *Scaphium* and *Sterculia* A). Growth by subdivision of existing initials is always present, and in the more advanced species it appears to predominate, sheath cells being formed only sporadically, and forming a very irregular layer as seen on the tangential section (*Cola*, *Sterculia* B, *Eribroma* and *Octolobus*). It is possible that the excessive growth of few rays is also a primitive feature, since the smaller rays seem to become the rule in some of the more advanced genera, but there is no evidence to confirm that this holds true throughout other families.

XI—CRYSTALLIFEROUS TISSUE

At the end of the last century investigations upon the occurrence of calcium oxalate in plants were very frequent, but the authors appear for the most part to have confined themselves to descriptive studies of the formation of crystal druses and calcium oxalate

crystals in leaves and meristems, and the formation of these crystals in woody tissues were usually omitted from their investigations.

There has been much discussion as to the importance of calcium oxalate in plant metabolism, and the conclusion usually arrived at is that it is almost always an excreted waste product, and that it has no active function in the plant tissues. Some authors—MEYER, COMBES, quoted by MILANEZ (1932)—suggest that the crystals are formed during the synthesis of proteins, but this would hardly account for their occurrence in the woody parts of plants. SCHIMPER (1890) has suggested that calcium oxalate is accumulated in the plant as the result of the withdrawal of phosphorus from the calcium phosphate of the crude sap, and that this accumulation would have fatal results if it were not rendered innocuous by combination with the oxalic acid left from respiration. Thus calcium and oxalic acid, both toxic to the plant, are withdrawn from the cell sap in the form of crystals, and are often further removed from all active contact with the plant by being surrounded by an impermeable membrane. HABERLANDT (1914, p. 533), too, considers that crystals are an excretory product, and suggests that their occurrence in the marginal strands of banded parenchyma may be merely putting them in a place where they can have least effect on the metabolism of the plant, since these strands abut only on one side on the parenchyma, and on the other touch the fibres, with which there is little communication. MILANEZ (1932) has recently stated that crystals of calcium oxalate in the wood of plants result from the activity of the cambial meristem, and has suggested that their frequent occurrence in terminal parenchyma (as in many of the *Caesalpiniaceae*) may be the result of the accumulation of excreta from the activity of the whole growing season. This appears to be a very probable explanation of the crystals in the chambered parenchyma, but does not seem equally applicable to the solitary crystals that are often found scattered irregularly through rays and parenchyma.

In the *Sterculiaceae* crystals appear to be absent altogether from certain genera, for example, *Pterocymbium* (a few solitary crystals were observed in one sample of *P. tinctorium* MERRILL), *Scaphium*, *Firmiana* and *Octolobus* (one sample only available). They were found to be constant in occurrence in both rays and parenchyma in *Pterygota*, *Brachychiton* and *Eribroma* (one sample only); sporadic in *Cola*, and present in the rays or parenchyma or both, in most species of *Sterculia*, *Tarrietia*, *Heritiera* and *Argyrodendron*. Crystalliferous fibres are present in *Sterculia* spp. and *Eribroma*.

The solubility of the crystals in HCl without effervescence and their solubility, though only after prolonged action, in copper sulphate, show them to be calcium oxalate. BARGAGLI-PETRUCCI (1903) mentions the occurrence of silica in the wood of *Heritiera littoralis* DRY. and *Sterculia* sp., but says that it is sporadic in occurrence; the author has not found silica in any of the samples of *Heritiera littoralis* examined.

The crystals are always surrounded by a membrane, and are isolated by it from the rest of the cell. WITTLIN (1896) describes the formation of this type of crystal sheath; it seems to be very similar to the Rosanoffian membrane that surrounds crystal

druses (ROSIANOFF 1865, 1867), but the trabeculae are lacking. WITTLIN states that "one is almost tempted to assume that every crystal is surrounded by a membrane, and that unenclosed crystals hardly ever occur". The author finds that this is true for all the genera of the Sterculiaceae examined, although there is very much difference in the thickness and visibility of this membrane. In some cases it is extremely thick and

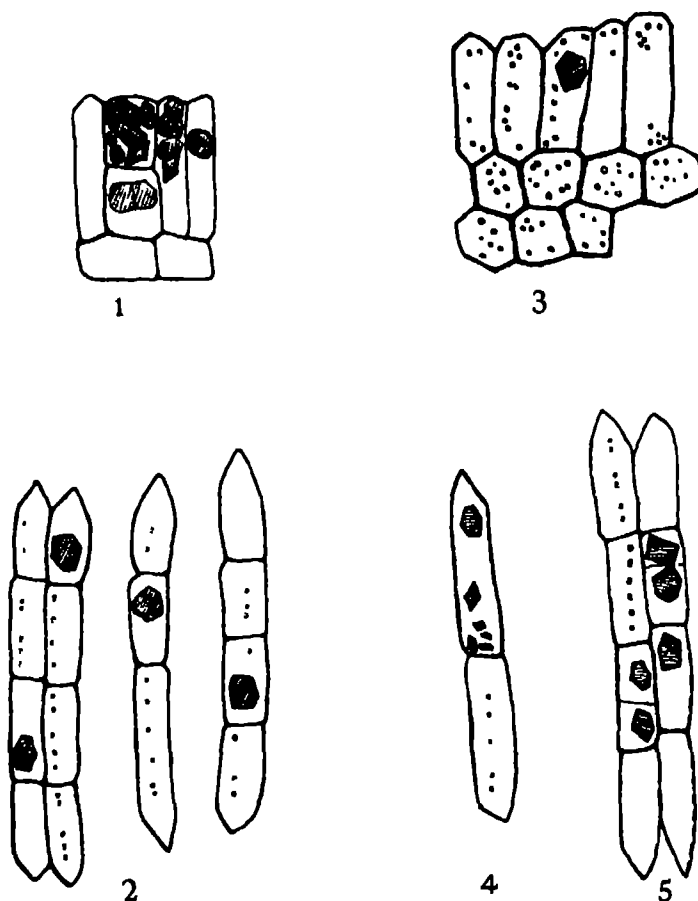


FIG. 21—Crystals in ray and parenchyma cells. 1, *Pterygota alata* ROXB.; crystals in upright ray cells. In the cell on the left the crystals are surrounded by thick membranes, while in the adjacent cells the membranes are very thin. 2, *Sterculia hypochra* PIERRE; solitary crystals in parenchyma strands. 3, *S. ornata* WALL.; solitary crystal in upright ray cell. 4, *S. hypochra* PIERRE; parenchyma strand, the upper cell of which contains several crystals. 5, *S. hypochra* PIERRE; parenchyma strands with paired crystals separated by thin septa. (All $\times 140$.)

has so closely invested the crystal as to give the impression that the crystal is still present, even after its solution in HCl; or it may be so thin as to be obscured by the crystal, and even after the crystal has been dissolved out it can only be seen with critical illumination. Some such variability in the thickness of the membrane is shown in all the figures illustrating this section, but especially in fig. 21 (1), which illustrates adjacent ray cells from *Pterygota alata* ROXB. containing crystals with both thin and thick membranes.

The crystals may occur either as large solitary crystals, or as groups of small crystals, in unchanged cells of the parenchyma or rays, or in specialized cells. The latter may be cells of the vertical parenchyma (the chambered parenchyma of the older writers), special septate or subdivided cells of the rays, or crystalliferous fibres.

Where the crystals lie free in unspecialized cells of the parenchyma or rays they are often conspicuous only in sections that have been prepared from unsoftened material, since they may be dissolved out by the action of the softening reagent, and leave only a very thin membrane, and no modification of the shape of the cell to tell of their presence. Figs. 21 (2) and (3) show such solitary crystals in parenchyma and ray cells respectively, while fig. 21 (4) illustrates a collection of smaller crystals within a single parenchyma cell. This figure may be compared with fig. 21 (5) which illustrates a pair of parenchyma strands from the same wood, where two crystals lying in a single parenchyma cell are seen to be separated from one another by a thin septum. This condition may be intermediate between the usual condition in this wood, and the more specialized types with true chambered parenchyma.

Chambered parenchyma strands occur scattered sparsely among the metatracheal parenchyma in *Heritiera* spp. and *Tarrietia* spp., but they are very common in *Argyrodendron trifoliatum* F. VON MUELL., *Eribroma klaineana* PIERRE, *Sterculia appendiculata* K. SCHUM., *S. elegantiflora* HUTCH. and DALZ., *S. oblonga* MAST., and *S. rhinopetala* K. SCHUM. In the last-named wood crystalliferous tissue is especially abundant, and special subdivided crystal cells occur in the rays as well as in the parenchyma (fig. 31, Plate 30). The crystalliferous tissue has two very characteristic features in all the above woods. The sheaths surrounding the crystals are always conspicuous, and it is extremely rare to find more than one crystal per cell.

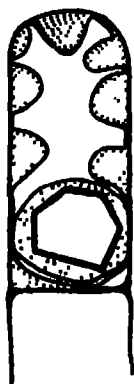


FIG. 22—*Sterculia quinqueloba*. K. SCHUM. Apical cell of crystalliferous parenchyma strand, showing crystal sheath and thickened wall. ($\times 500$.)

The extra deposition of thickening in crystalliferous cells is not always confined to the crystal sheath, but also affects the wall of the cell, which may become much thicker than is usual for parenchyma (fig. 22). This is also illustrated in figs. 23 and 24, which show that the thickening is laid down more on one side of the cell than on the other. This particular manner of thickening is very noticeable in all the *Sterculia* species that have chambered parenchyma. They all have broad bands of parenchyma, and the crystalliferous strands are confined to the margins of the bands, and the thickening is always more marked on the side that is towards the fibres. Sometimes the crystal membrane itself, as well as the cell wall, is much thinner towards the parenchyma. Figs. 23 (1-5) and 24 (1-3) and (6-7) show such strands viewed from the tangential direction; the thickening of both the wall and the crystal sheath is more regular, and usually thinner, on the radial than on the tangential walls.

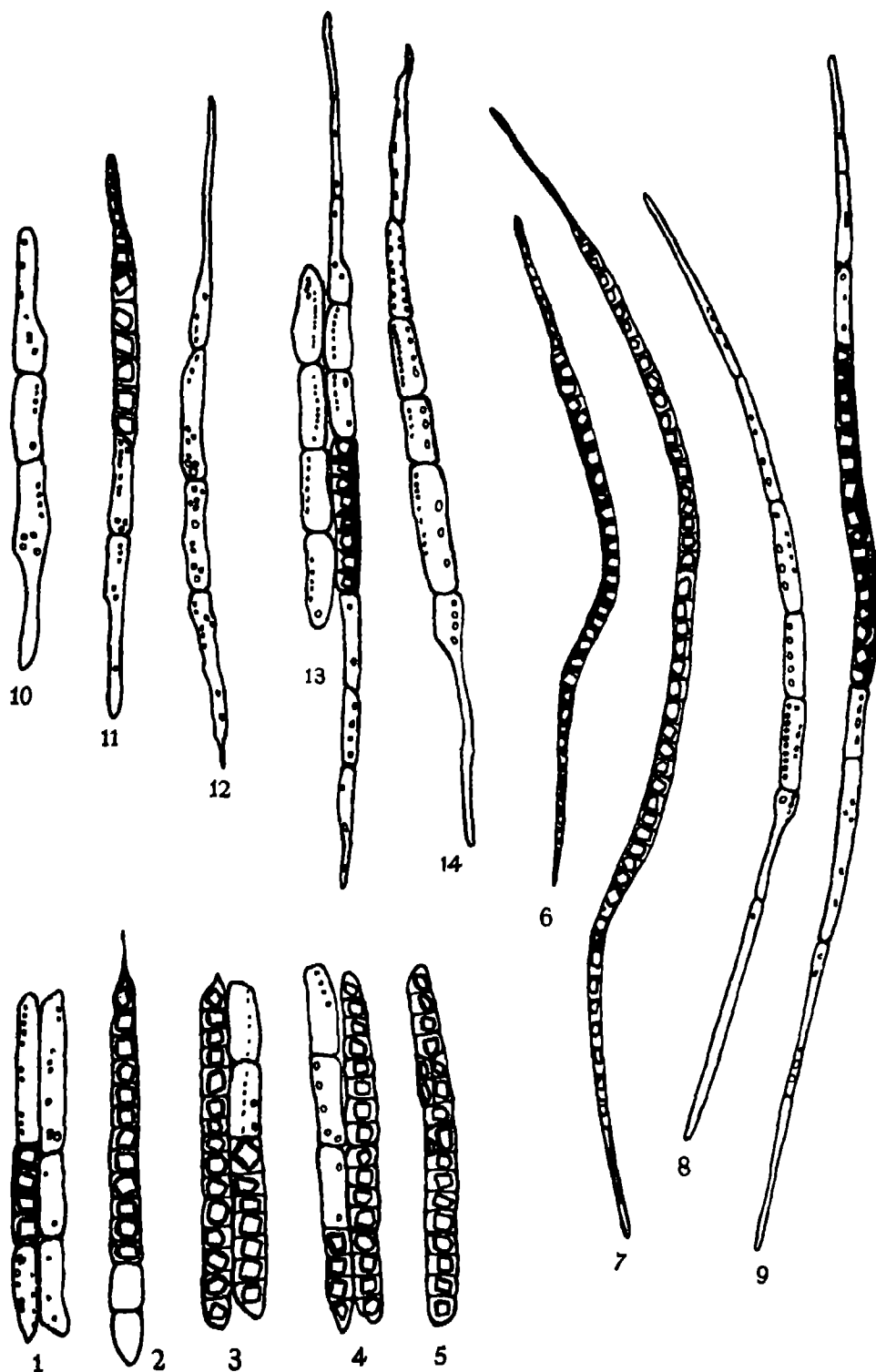


FIG. 23—*Sterculia oblonga* MAST. Crystalliferous tissue. 1-5, chambered parenchyma strands, containing solitary crystals. 6 and 7, crystalliferous fibres. 8-14, elongated strands, subdivided by cell walls, and occasionally also septate; the septate portions containing crystals. (All $\times 140$.)

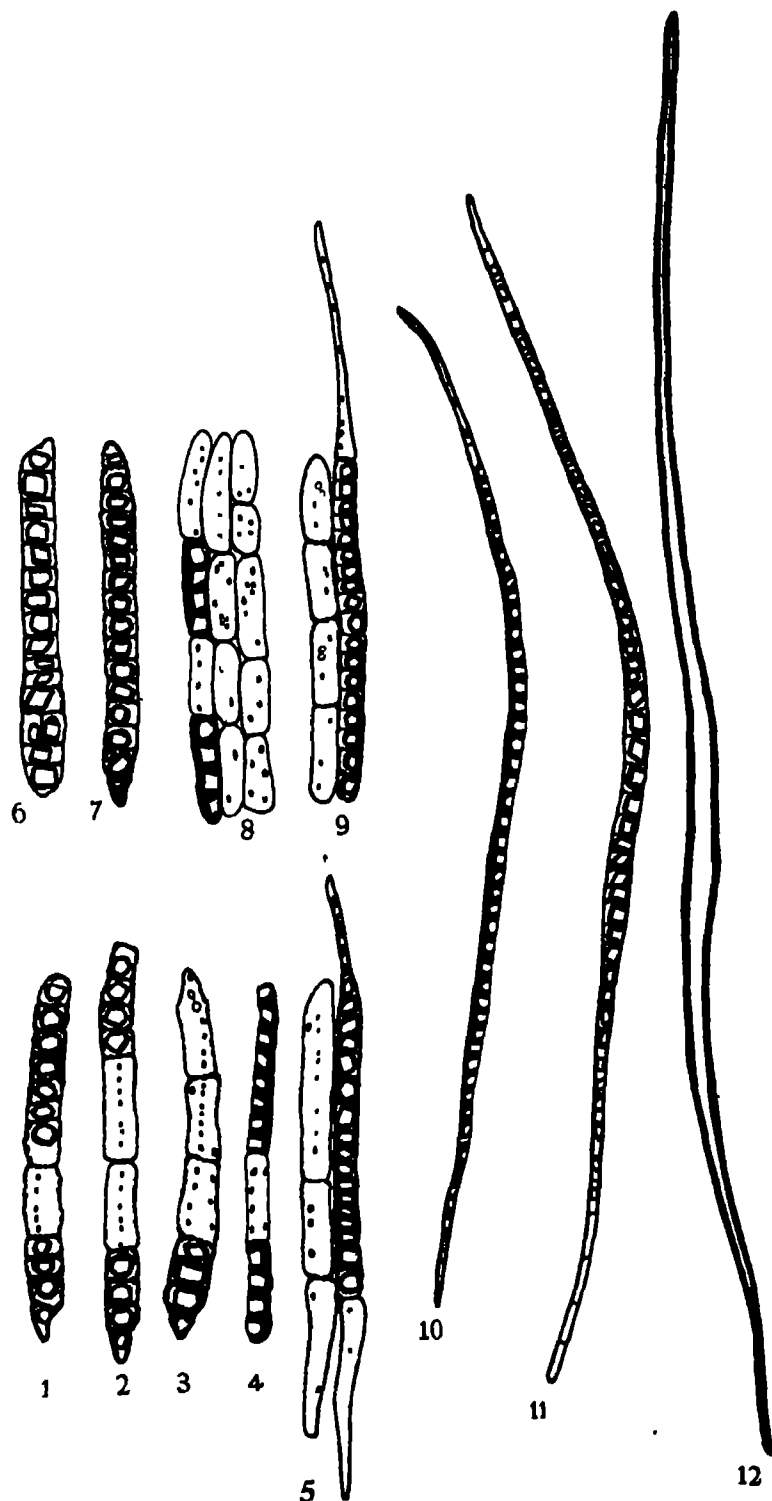


FIG. 24—*Sterculia elegantiflora* HUTCH. and DALZ. Crystalliferous tissue. 1-4 and 6-8, chambered parenchyma strands, containing solitary crystals. 5 and 9, elongated strands, subdivided by cell walls, and also septate; the septate portions containing solitary crystals. 10-12, libriform fibres; 10 and 11, containing crystals. (All $\times 140$.)

MILANEZ (1932) has described this type of parenchyma very fully, and has given names—hemi-crystalliferous, holo-crystalliferous, etc.—to the different parenchyma strands. Such terms appear to the author to be both unnecessary and confusing. The cells are essentially the same in all cases; they are parenchyma cells that have been further subdivided to contain crystals, and the distinction drawn by MILANEZ merely refers to cases where one, two, or more cells of a parenchyma strand are so divided. Figs. 23 (1–5), and 24 (1–4) and (6–8) illustrate such series of crystalliferous parenchyma strands from *Sterculia oblonga* MAST. and *S. elegantiflora* HUTCH. and DALZ. respectively, and show that in each instance the cells of the strand have been regularly divided into four crystalliferous cells. Sometimes the subdivision is by walls and sometimes by septa; in the former case the pits in the dividing wall can be seen. Owing to the thickening of the cell wall that commonly occurs in these cells, and to the thick membrane that surrounds the crystal, the distinction between wall and septum is often difficult to see, but may sometimes be made more easily on slides of macerated material. The action of the macerating fluid causes the strands of parenchyma to disintegrate into their component cells, but the cells themselves have not disintegrated in the same way, as they are only divided by septa, and each cell still consists of four compartments.

MILANEZ (1932) states that the “crystal fibre of certain authors is almost always a simple series of crystalliferous parenchyma cells”, and adds that he has not succeeded in finding crystals of calcium oxalate in authentic wood fibres. The author cannot agree with this statement. In the *Sterculia* species that have chambered parenchyma, undoubted crystalliferous fibres are often present. Stages intermediate between parenchyma and fibres can be found in *S. elegantiflora* and *S. oblonga*. In the latter, strands of four or more cells that are parenchymatous in nature can often be found; they are thin-walled, and are subdivided by walls and not by septa, and have simple pits similar to those of the normal parenchymatous cells, but the series does not conform to the definition of a wood parenchyma strand, since it is often considerably longer than the cambial initial from which it was derived (International Association of Wood Anatomists 1933). In the *Sterculia* species illustrated in figs. 23 and 24, the length of the parenchyma strand probably corresponds very closely with the length of the cambial initial, and shows little variation from strand to strand; such strands are illustrated in fig. 23 (1) and (13), and in fig. 24 (8) and (9). The chambered strands are usually the same length, and again there is little variation in length between them. But there are also strands that are half-parenchymatous and half-fibrous; such strands occur also quite commonly in other woods. In *S. oblonga* and *S. elegantiflora* there are also very long strands which still retain the form of parenchyma, but which seem to have undergone some, often considerable, elongation. Such strands are illustrated in figs. 23 (8–14), and fig. 24 (5) and (9). The cells are divided by walls and not by septa, and the pitting corresponds to that usually found in parenchyma, and not to that found in fibres. Usually some of these long parenchyma strands are subdivided to

contain crystals (fig. 23 (9), (11) and (13)). Crystalliferous strands that are much longer than the parenchyma strands are illustrated in fig. 23 (6) and (7) and fig. 24 (10) and (11). These are subdivided, and contain crystals throughout their length, and are probably the "crystal fibres" of other writers (HARTIG, quoted by HABERLANDT 1914). It is difficult to see whether the cells are divided by walls or by septa, but from their appearance in slides of macerated material, the latter seems more probable, as they seldom disintegrate during maceration. They are usually slightly shorter than the longest fibres, but approximate more closely to them in length than to the parenchyma strands. In fig. 24 (11) and (12), two fibres are shown side by side for comparison, one a crystalliferous fibre, the other a normal libriform fibre of average length. This is the only occurrence of septation of the fibres within the Sterculiaceae.

It seems doubtful whether any phylogenetic importance can be attached to the occurrence of crystals. Solitary crystals in unspecialized cells are certainly sporadic in occurrence, and may be present in some samples of a species, but not in others. But the author has found, both in the Sterculiaceae and in other families that she has examined, that chambered parenchyma is much more constant in its occurrence. Until a review of the distribution of chambered parenchyma, and that of crystalliferous fibres in the different families of dicotyledons has been made, it is impossible to decide whether they are a primitive feature or a specialized one. Within the Sterculiaceae chambered parenchyma and crystalliferous fibres are confined to the more advanced genera, though they are not universal in these—for example, they are absent altogether from *Cola*; and the absence of crystals from the wood of *Pterocymbium* and *Scaphium* cannot be considered as a sign of primitive structure, as they are also absent from species of *Cola* and *Firmiana* and from the sample of *Octolobus* examined. Both isolated crystals and chambered parenchyma are however very useful diagnostic features, and may serve to establish the affinities of doubtful genera.

XII—THE GENERA

In the following account of the genera the author has not attempted to give a detailed description of each genus, but rather to pick out the particular features by which each can be distinguished from the rest. Details of the elements and tissues have already been given for each genus, and all that will now be attempted will be to show how far the present classification of the species is consistent with the wood anatomy. Several of the species have already appeared in different genera at different times, and though some of them still remain a puzzle, it is possible that the wood anatomy may in some cases suggest the appropriate relationships.

*The tribe Sterculineae (EDLIN 1935)*1—*Brachychiton* SCHOTT. and ENDL.

B. acerifolius A. CUNN.; *B. discolor* F. VON MUELL.; *B. rupestris* K. SCHUM.

The first two species of this genus appear to be indistinguishable from species of *Sterculia* A, except as regards vessel size and distribution. The species have all at one time or another been included in the genus *Sterculia*, and there appears to be no adequate reason for separating them, on the grounds of wood anatomy; they have in common, the arrangement of the parenchyma, and the type of vessel-parenchyma pit-pairs.

Brachychiton rupestris K. SCHUM. (fig. 32, Plate 30) is different from all the other material of the Sterculiaceae examined, in having large cavities in the parenchyma. These are possibly for water storage, and the name "Australian bottle tree" may owe its origin to this, or to the curious shape of the trunk. Owing to these cavities the wood is extremely spongy, and of an irregular texture, the cavities alternating with masses of harder fibrous tissue. Apart from this peculiarity it has much in common with the species of *Sterculia* B, having very short vessel members, broad bands of parenchyma, four cells per parenchyma strand, with intercellular spaces between the cells, and chambered crystalliferous parenchyma. Only one small sample of this species was available, and it has been considered advisable to omit it from the table of measurements in view of its somewhat anomalous structure. Such measurements as are available, however, show much closer agreement with *Sterculia* B than with the other species of *Brachychiton*.

The author finds that, on anatomical evidence, there is little distinction between the genera *Brachychiton* and *Sterculia*; for *Brachychiton acerifolius* and *B. discolor* agree closely with the section A of that genus, and *B. rupestris* with the section B. There appears also to be very little morphological difference between *Brachychiton* and *Sterculia*, and BENTHAM and HOOKER (1867) did not consider the differences sufficient to separate the two genera.

2—*Cola* SCHOTT.

C. acuminata SCHOTT. and ENDL.; *C. buntingii* BAK. f.; *C. caricifolia* K. SCHUM.; *C. chlamydanthia* K. SCHUM.; *C. cordifolia* R. BR.; *C. heterophylla* SCHOTT. and ENDL.; *C. lateritia* K. SCHUM.; *C. laurifolia* MAST.; *C. lepidota* K. SCHUM.; *C. mirabilis* A. CHEV.; *C. nitida* A. CHEV.; *C. togoensis* ENGL. and KRAUSE; *C. verticillata* STAFF.

This genus appears to be very homogeneous, and the species are often indistinguishable from one another. They are also very similar to species of *Sterculia* B, from which they can only be distinguished by the absence of chambered crystalliferous parenchyma. Solitary crystals appear sporadically in the rays and parenchyma of a few species, but neither crystalliferous fibres nor chambered parenchyma have been observed in any species.

3—*Eribroma* PIERRE.*E. klaineana* PIERRE,

Only one sample of this genus was available. The species was previously placed in the genus *Sterculia*, and from its wood anatomy there appears no reason why it should not be included in this genus. All the features measured fell within the range for the different species of *Sterculia* B, though they are below the average. Some of the species of *Sterculia* B cannot be distinguished from *Eribroma*. The presence of chambered crystalliferous parenchyma and crystalliferous fibres in the wood of *Eribroma* serve to distinguish this genus from *Cola*, which it otherwise resembles. Neither HARMS (DALLA TORRE and HARMS 1900-7) nor THONNER (1915) recognizes *Eribroma* as a distinct genus, but both sink it in *Sterculia*.

4—*Firmiana* MARSIGLI.*F. barteri* K. SCHUM.; *F. colorata* R.BR.; *F. fulgens* WALL.; *F. populifolia* TERRAC.; *F. simplex* W. F. WIGHT.

The species of this genus are very similar to one another in structure, with the exception of *F. simplex* W. F. WIGHT, which differs only in being ring-porous.

The bands of parenchyma that occur in this genus are often apparently composed mainly of paratracheal parenchyma, and may occasionally be entirely confluent in nature.

Firmiana can be distinguished from the other genera that have broad bands of parenchyma by the absence of crystals (occasional crystals were observed in one sample), and by the unilaterally compound vessel-parenchyma pit-pairs.

In 1932 the author (CHATTAWAY 1932) suggested that *Sterculia pallens* WALL. was related to *Firmiana*, through *F. wallichii* R.BR. This species, and also *F. affinis* MAST., have now been transferred to the genus *Scaphium*, with which they have much in common. *Sterculia pallens*, however, closely resembles some of the other species now remaining in the genus *Firmiana*, having parenchyma that is apparently confluent, and it shares with them the unilaterally compound vessel-parenchyma pit-pairs. The author suggests that this species of *Sterculia* should be transferred to *Firmiana* (fig. 33, Plate 30).

In a recent paper, RIDLEY (1934) transferred *F. colorata* R.BR., *F. fulgens* WALL. and *Sterculia pallens* WALL. to another genus, *Erythropsis*. These woods cannot be distinguished from one another, or from the other species of *Firmiana*, and the wood anatomy suggests that the correct solution lies in transferring *Sterculia pallens* to *Firmiana*, rather than in the use of another generic name—*Erythropsis*—for all three woods.

5—*Octolobus* WELW.*O. spectabilis* WELW.

Only one sample of this species was available. It is indistinguishable from *Cola*, except for the extremely small intervacular pit-pairs.

6—*Pterocymbium* R.BR.

P. javanicum R.BR.; *P. tinctorium* K. SCHUM.

The two species included in this genus are indistinguishable from one another in their wood structure. They are very similar in type to *Brachychiton* and *Sterculia* A, from which they can, however, be separated by the absence of crystals, and by the vessel-parenchyma pit-pairs. These are similar in size and shape to the intervacular pit-pairs, and are quite distinct from the large simple pits to parenchyma cells that characterize the vessels of *Brachychiton* and *Sterculia* A.

The strands of metatracheal parenchyma are commonly one- or two-celled, and owing to the thinness of the fibre walls the distinction between the fibres and parenchyma, as seen on the cross-section, is never very sharp.

BENTHAM (BENTHAM and HOOKER 1867) found that there was occasionally a tendency to the hermaphrodite condition in *Pterocymbium javanicum*, and suggested that this was a primitive condition. The present author has already pointed out that *Pterocymbium* has certain primitive features in the wood.

7—*Pterygota* ENDL.

P. alata ROXB.; *P. macrocarpa* K. SCHUM.; *P. kamerunensis* K. SCHUM.

The species of *Pterygota* examined are rather similar in their general features to *Cola* and *Sterculia* B. The genus has been considered a synonym for *Sterculia*, but is maintained as a distinct genus by DALLA TORRE and HARMS (1900-7). The main difference from *Cola* lies in the abundance of solitary crystals in the rays and parenchyma of all the material examined. The absence of chambered parenchyma and crystalliferous fibres serves to distinguish *Pterygota* from some, though not all, of the species of *Sterculia* B.

8—*Scaphium* ENDL.

S. affinis MAST.; *S. linearicarpum* RIDL.; *S. macropodium* MIQ.; *S. wallichii* R.BR.

The species of *Scaphium* examined have almost all at one time or another been considered as members of some other genus. *S. macropodium* has been transferred from *Sterculia*; *Scaphium wallichii* was once *Sterculia scapigera*, and was thence transferred to *Firmiana* as *F. wallichii* and finally to *Scaphium*, where it appears to fit best, at least so far as the anatomy is concerned; *S. affinis* has also been transferred from *Firmiana*, and in this case too the wood appears to resemble the other species of *Scaphium* much more than any species of *Firmiana* examined.

The genus differs from *Sterculia* mainly in the absence of metatracheal parenchyma. The paratracheal parenchyma is often rather scanty, sheathing the vessels, but occasionally it is distinctly aliform (fig. 34, Plate 30). This is interesting in view of the affinity with *Firmiana*. There is obvious confusion between the two genera, as is shown by the various changes that have been made in their classification. In both *Scaphium*

and *Firmiana* the parenchyma, though banded in the latter, is probably paratracheal, and it is possible that the two genera represent a somewhat different line of development from the rest of the family.

9—*Sterculia* L.

Sterculia A. Metatracheal parenchyma predominantly in lines one cell wide (fig. 35, Plate 31).

S. angustifolia ROXB.; *S. cariboea* R.BR.; *S. carthagenensis* CAV.; *S. columbiana* SPRAGUE; *S. crassiramea* MERRIL; *S. foetida* L.; *S. harmanda* PIERRE; *S. hypochra* PIERRE; *S. javanica* R.BR.; *S. macrophylla* VENT.; *S. montana* MERRIL; *S. oblongata* R.BR.; *S. ornata* WALL.; *S. parviflora* ROXB.; *S. philippinensis* MERRIL; *S. recordiana* STANDL. (STANDLEY 1935); *S. rubiginosa* VENT.; *S. spangleri* R.BR.; *S. tragacantha* LINDL.; *S. urceolata* SMITH; *S. villosa* ROXB.

There is great similarity in general features between the species of this section of the genus. Occasional samples may show sporadic bands of parenchyma, and occasionally abnormalities such as wide regions of very thin-walled tissue may be found, but the parenchyma is usually paratracheal, in wide sheaths around the vessels, and metatracheal, in lines one cell wide. These woods are often indistinguishable from *Brachychiton*. They often have large simple pits from vessels to parenchyma cells. Crystals are often present, in the rays and parenchyma, but are usually solitary, in unspecialized cells, and the crystal sheath is usually rather thin.

Sterculia B (fig. 36, Plate 31). Metatracheal parenchyma and paratracheal parenchyma often indistinguishable, predominantly in broad bands three or four cells wide.

S. appendiculata K. SCHUM.; *S. blancoi* ROLFE; *S. blumei* G. DON.; *S. cinerea* A. RICH.; *S. coccinea* ROXB.; *S. elegantiflora* HUTCH. and DALZ.; *S. oblonga* MAST.; *S. pallens* WALL.; *S. quinqueloba* K. SCHUM.; *S. rhinopetala* K. SCHUM.; *S. urens* ROXB.

It has already been suggested that the wood of *S. pallens* resembles that of *Firmiana* more than that of the other species of *Sterculia* B. As RIDLEY (1934) has already suggested affinities with *Erythropsis* (indistinguishable from *Firmiana*), the author suggests that it should be transferred from *Sterculia*.

The other species of *Sterculia* that have broad bands of parenchyma are often very similar in general structure to *Cola*, from which they can be distinguished by the presence of chambered parenchyma. Four species, however, *Sterculia cinerea*, *S. coccinea*, *S. quinqueloba* and *S. urens*, are sometimes intermediate in type between the two sections of the genus, and they may occasionally have a considerable amount of metatracheal parenchyma scattered between the bands, either as isolated strands or as short lines. The vessel-parenchyma pit-pairs may occasionally be simple (constantly in *S. quinqueloba*), and chambered parenchyma strands and crystalliferous fibres are absent. They thus appear to be borderline cases, and it is difficult to assign them to either section of

the genus with certainty. On the whole, in the majority of samples examined, there appears to be more similarity to this section of the genus than to section A.

The tribe Tarrietineae (EDLIN 1935)

1—*Argyrodendron* F. v. MUELL.

A. actinophyllum (MOORE) EDLIN; *A. trifoliatum* F. v. MUELL.; *A. actinophyllum* var. *peralatum* (F. M. BAILEY).

The genus *Argyrodendron* was founded by F. VON MUELLER, for *Tarrietia argyrodendron*. This genus was referred back to *Tarrietia* by BENTHAM and HOOKER (1867), but has been revived by EDLIN (1935) to include *T. argyrodendron* and *T. actinophylla*. These two species are quite distinct anatomically from the other species of *Tarrietia* examined, having broad bands of parenchyma, whereas the species of *Tarrietia* have narrow lines, and it seems proper that the two species should be kept in a separate genus.

The author has also received material from Australia bearing the designation *T. argyrodendron* var. *peralata*. From the wood structure these specimens are clearly allied to *Argyrodendron*, and the material undoubtedly represents *Tarrietia trifoliata* var. *peralata* F. M. BAILEY. The author suggests that as it cannot be separated from *Argyrodendron* from the same source, *A. actinophyllum* var. *peralatum* is a better designation for it.

2—*Heritiera* AIT.

H. elata RIDLEY; *H. fomes* SYME.; *H. littoralis* DRY.; *H. macrophylla* WALL.

The genus *Heritiera* is very homogeneous, and the species are very similar anatomically. They can be distinguished from *Argyrodendron* by the narrow lines of metatracheal parenchyma one cell wide, and from *Tarrietia* by the rays, in which there is often only a very slight distinction between marginal and central cells, by the absence of sheath cells and by the occasional presence of chambered strands in the metatracheal parenchyma. This feature and the occurrence of dark gum-like deposits in the rays and parenchyma distinguish species of *Heritiera* from the species of *Sterculia* A. It is probable that *Heritiera* represents a more advanced type of structure than most woods with narrow lines of metatracheal parenchyma, and possibly an advance in ray structure, and in the parenchyma strand itself, has been accompanied by a lag in the type of parenchyma and its distribution.

3—*Tarrietia* BL.

T. cochinchinensis PIERRE; *T. javanica* BL.; *T. perakensis* KING; *T. simplicifolia* MAST.; *T. sumatrana* MIQ.; *T. sylvatica* MERRIL; *T. utilis* SPRAGUE.

The species now placed (EDLIN 1935) in the genus *Tarrietia* are very similar to one another anatomically. *T. utilis* SPRAGUE was formerly placed in the genus *Heritiera* as *H. utile*. The woods of *Heritiera* and *Tarrietia* have many points of similarity, especially

when seen in cross-section but the rays of *T. utilis* are markedly heterogeneous, and often have a very conspicuous sheath of erect marginal cells, while the rays of *Heritiera* often have little distinction between marginal and central cells, and are almost always without sheath cells. The wood anatomy thus confirms the transference to *Tarrietia*.

Suggested changes

The following changes in classification are suggested:

Sterculia pallens WALL. to be transferred to *Firmiana*; *Brachychiton* and *Eribroma* again to be sunk in *Sterculia*; the genus *Sterculia* to be subdivided into two subgenera.

The rearrangement of these genera is reflected in the dimensions of the elements. The alterations in the mean values for vessel-member length, vessel diameter and fibre length are given below.

Vessel-member length. *Sterculia* A is altered from 441 ± 55.2 to $446 \pm 54.8 \mu$; *Sterculia* B from 353 ± 51.4 to $337 \pm 32.8 \mu$; *Firmiana* from 414 ± 99.5 to $420 \pm 95.5 \mu$. The changed position of *Sterculia pallens* WALL. from *Sterculia* B to *Firmiana* makes a significant difference to the mean lengths of the former genus, but not to that of the latter. This is not unexpected, for *Sterculia pallens* is the only species in *Sterculia* B with vessel members much above the average length for that group, but they are very little different from the average length for *Firmiana*, and the addition of this species to the genus *Firmiana* does not make a significant difference to the mean length of the genus.

Vessel diameter. *Sterculia* A is altered from 187 ± 31.0 to $176 \pm 37.4 \mu$; *Sterculia* B from 156 ± 25.0 to $155 \pm 25.6 \mu$; *Firmiana* from 153 ± 29.5 to $153 \pm 29.7 \mu$. The addition of the specimens of *Brachychiton* to *Sterculia* A reduced the average diameter by a significant amount. The vessels of *Brachychiton* were much smaller than was expected for this type of wood, but as this was the only feature in which *Brachychiton* differed from *Sterculia* A it was not considered sufficient to warrant keeping the two genera separate.

Fibre length. *Sterculia* A is altered from 2029 ± 334 to $2009 \pm 335 \mu$; *Sterculia* B from 2017 ± 317 to $2008 \pm 326 \mu$; *Firmiana* from 1939 ± 226 to $1920 \pm 221 \mu$. None of these changes is significant.

XIII—CONCLUSION

The most interesting feature of the wood of the Sterculiaceae lies in the unusual relations of the metatracheal and paratracheal parenchyma, and in the development of the rays. The latter show two different ways of solving the problem of spreading the ray tissue through the wood, neither of them apparently leading on to any further advance, but probably representing side-lines of specialization, since the main line of development is towards the formation of small rays. The splitting up of large rays would have the effect of forming numerous small rays, if the rays so formed did not

grow and split and grow again. As it is there is always a large number of big rays present in the wood, and the trend of development towards smaller rays is not achieved in this way. The only genus in which there appears to be any advance along the main line of the phylogenetic sequence established by KRIBS is *Heritiera*, in which the rays are reduced in size, and sometimes approach the homogeneous condition.

The most characteristic anatomical features of the Sterculiaceae are as follows: Both paratracheal and metatracheal parenchyma occur in the same wood, either independently as sheaths round the vessels and narrow metatracheal lines, or combined as broad bands. The parenchyma and small rays are storeyed, but the larger rays are higher than, and independent of, the storeys, and are surrounded by sheath cells. Other characters that are consistent throughout the family are vessels with simple perforation plates, alternate intervascular pitting, libriform fibres that are never septate, and the absence of tracheids. Within the family, the genera can be distinguished by the type of parenchyma, the size of the intervascular and vessel-parenchyma pitting, the grouping of the vessels, and the occurrence of chambered parenchyma and crystalliferous fibres, or solitary crystals. Within the genera it is seldom possible to distinguish the species, except *Firmiana simplex* which is ring-porous, and *Brachychiton rupestris* which has anomalous structure.

The family Sterculiaceae is similar in many respects to the other families of the Malvales—Bombacaceae, Malvaceae and Tiliaceae; it is most easily confused with the Bombacaceae. In the Bombacaceae there is only one genus with broad bands of parenchyma—*Catostemma*—and the vessel arrangement and the purely paratracheal nature of the bands serves to distinguish it from those Sterculiaceae which have broad bands of parenchyma. The other genera of the Bombacaceae can be distinguished from the Sterculiaceae which have narrow lines of parenchyma by the presence of tile cells (Durioideae), by the more continuous lines of metatracheal parenchyma, which often alternate with great regularity with layers of fibres one cell wide, by the rather scanty development of the paratracheal parenchyma and by the absence, or irregular development, of sheath cells. In the Sterculiaceae only *Brachychiton* and *Sterculia* A. regularly have large vessel-parenchyma pit-pairs, but in the Bombacaceae they are present in all the genera that are without tile cells, except *Maxwellia* and *Montezuma*.

EDLIN (1935), basing his conclusions upon the floral morphology of the different families, was led to the conclusion that the Tiliaceae is the most primitive family of the Malvales, and he suggested that the three families Bombacaceae, Buettneriaceae and Sterculiaceae have been derived from the Tiliaceae upon mutually independent lines. He further suggested that the Malvaceae have arisen from the Bombacaceae as a result of further specialization.

The author has not studied the other families of the Malvales in as much detail as the Sterculiaceae, but examination of the wood appears to confirm the view that the Buettneriaceae, Bombacaceae and Sterculiaceae may have been derived from the Tiliaceae, but have developed along independent lines. EDLIN suggested that the

unisexual and apetalous flowers and apocarpous ovary that separate the Sterculiaceae so clearly from the other Malvales represent a side-line of evolutionary development, and do not lead to other alliances. The author has already suggested that the ray development in this family represents such a side-line, and it is also possible that the combination of paratracheal and metatracheal parenchyma in broad bands, such as occur in *Cola*, *Sterculia* B, etc., represents the end of a series that does not give rise to any other forms. Bands of parenchyma are found occasionally in the other families of the Malvales, but these are much less regular, and appear to be entirely paratracheal.

EDLIN suggests that this family has been derived from the Tiliaceae through the genus *Christiana*. This genus differs from the Sterculiaceae, particularly in regard to the wood parenchyma and rays, but it is not easy to be certain whether these differences render such a derivation impossible. Diffuse parenchyma is absent from *Christiana*, and as this is probably a primitive feature, it seems unlikely that it should originate in woods derived from *Christiana*. The rays in *Christiana* are smaller, and though of the same general type (heterogeneous II), probably represent a more advanced type than those found in the Sterculiaceae. It seems more likely therefore that if the Sterculiaceae are derived from the Tiliaceae they have come through some other genus, perhaps no longer living. Until more is known of the development of the different tissues, the evidence supplied by the wood is insufficient to decide whether the Sterculiaceae have been derived from the Tiliaceae, or whether both have come from a common ancestor.

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XIV—SUMMARY

1—The Sterculiaceae must be considered a rather advanced family; the genera all have storeyed structure, vessel members less than 550μ long, simple horizontal perforation plates, and alternate pitting.

2—The vessels vary from narrow to wide in the different genera, and vessel diameter appears to have little systematic significance. A positive relation was observed between diameter and vessel-member length, but there is no relation between vessel-member length and vessel distribution.

3—The size and frequency of the intervacular pitting is constant throughout each genus, with the exception of *Sterculia*, and the genera with the longest vessel members have the largest pits. Large simple pits between vessels and parenchyma cells, that occur in *Brachychiton* and *Sterculia* species, have been studied in detail.

4—The fibres are libriform and non-septate throughout the family, except for the crystalliferous fibres in *Eribroma* and *Sterculia* species. There is a negative relation between vessel-member length and the relative amount the fibres have extended during differentiation, the greatest relative extension occurring in the woods with the shortest vessel members.

5—The different forms of parenchyma are discussed, and the anatomical characters of the cells. The most advanced type of parenchyma in the family appears to be broad bands that include both metatracheal and paratracheal parenchyma. This form appears to represent the ultimate stage of two separate lines of development, one represented by the series diffuse—narrow metatracheal lines—broad metatracheal bands, and the other by the series vasicentric—aliform—confluent.

6—The rays show very little difference in type throughout the family, and cannot be used, except in the case of *Heritiera*, to separate the genera, but sheath cells are slightly more regular in the woods with the longest vessel members and narrow lines of parenchyma, than in those with shorter vessel members and broad bands of parenchyma. The occurrence and development of sheath cells and the increase in the number of rays by splitting up of the larger rays has been studied in detail.

7—Crystals are of frequent occurrence throughout the family, and appear to have little phylogenetic significance; they are, however, useful diagnostic features. Chambered parenchyma and crystalliferous fibres have been studied in *Eribroma* and *Sterculia* species.

8—The taxonomic position of the genera is discussed, and the following changes in classification suggested: *Sterculia pallens* WALL. to be transferred to *Firmiana*; *Brachychiton* and *Eribroma* again to be sunk in *Sterculia*; and the genus *Sterculia* to be subdivided into two subgenera.

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DESCRIPTION OF PLATES

PLATE 29

FIGS. 25-27—Distribution of parenchyma. FIG. 25—*Pterocymbium tinctorium* K. SCHUM., scattered cells and short tangential lines. FIG. 26—*Sterculia carthagenensis* CAV., abundant paratracheal parenchyma, and narrow metatracheal lines. FIG. 27—*Cola caricifolia* K. SCHUM., broad metatracheal bands. (All $\times 33$.)

FIGS. 28-30—Types of parenchyma cell. FIG. 28—*Pterocymbium javanicum* R. BR., two cells per strand, without intercellular spaces. FIG. 29—*Sterculia recordiana* STANDL., two to four cells per strand, without intercellular spaces. FIG. 30—*Sterculia appendiculata* K. SCHUM., four or more cells per strand; cells with rounded corners and intercellular spaces. (All $\times 155$.)

FIG. 25

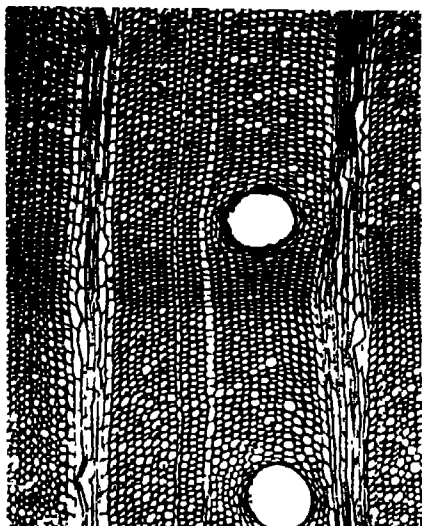


FIG. 26

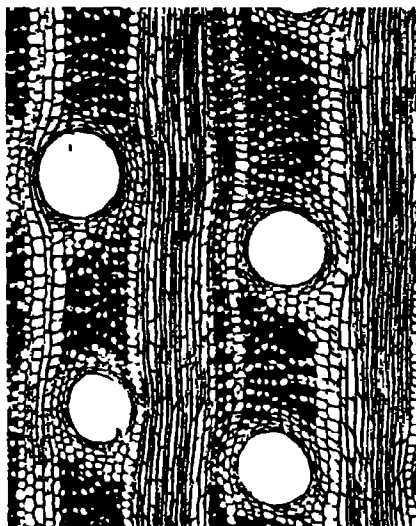


FIG. 27

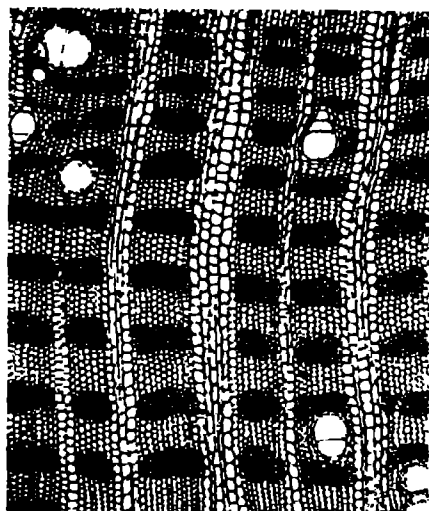


FIG. 28



FIG. 29

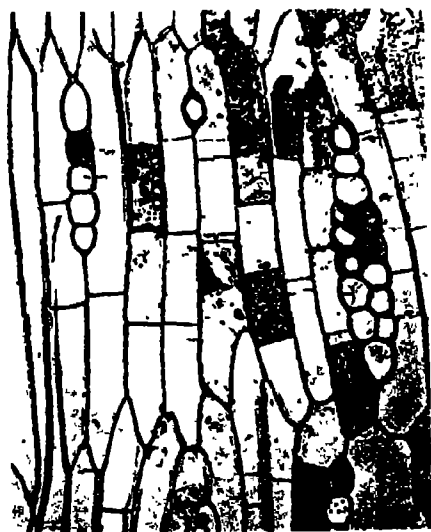


FIG. 30



PLATE 30

FIG. 31—*Sterculia rhinopetala* K. SCHUM. Radial section; solitary crystals in rays, parenchyma and fibres. ($\times 175$.)

FIG. 32—Transverse section of *Brachychiton rupestris* K. SCHUM.; showing broad bands of parenchyma and large cavities. ($\times 10$.)

FIG. 33—Transverse sections of: *a*, *Sterculia pallens* WALL.; *b*, *Firmiana fulgens* WALL.; showing the similarity of structure in the two woods. ($\times 33$.)

FIG. 34—Transverse sections of *Scaphium* spp. *a*, *S. macropodium* MRO. parenchyma terminal and vasicentric; *b*, *S. wallichii* R.Br. parenchyma terminal and aliform, occasionally confluent. ($\times 33$.)

FIG. 31



FIG. 32



FIG. 33
a

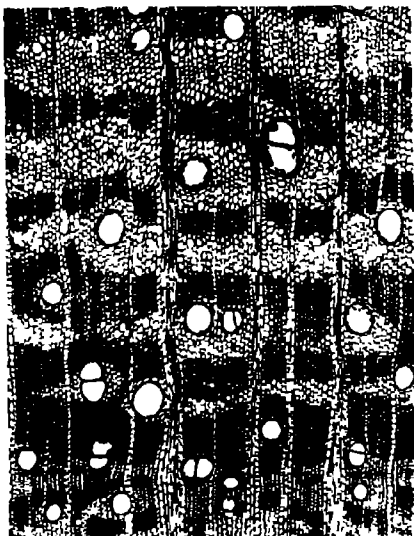


FIG. 33
b

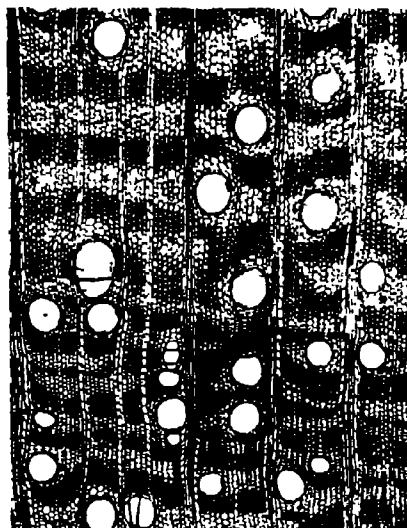


FIG. 34
a



FIG. 34
b

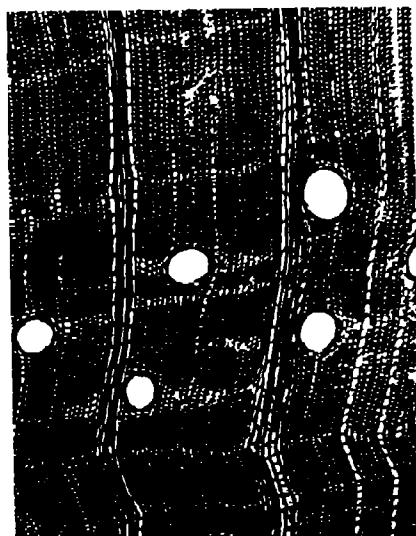
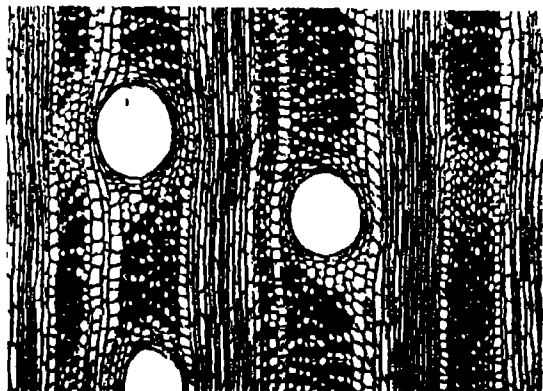
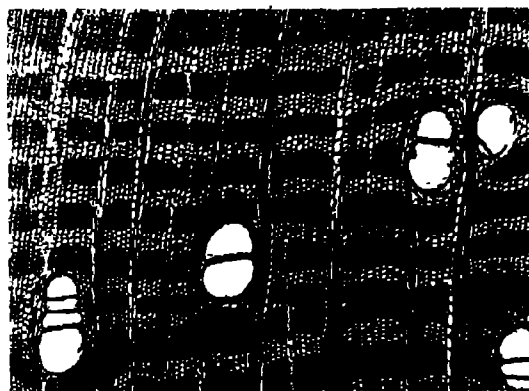


PLATE 31

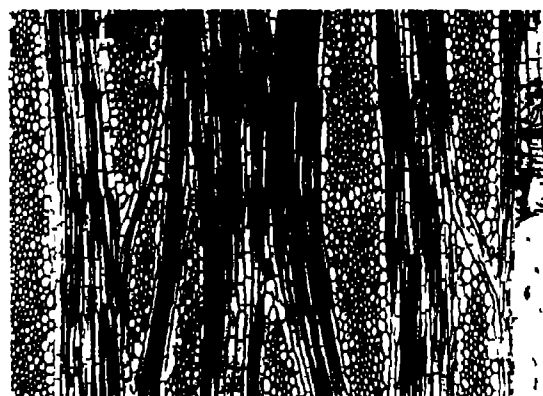
FIGS. 35, 36—Comparison of *Sterculia* A and *Sterculia* B. 1, transverse sections: A, *S. carthagenensis* CAV. B, *S. blancoi* ROLFE. 2, tangential sections: A, *S. recordiana* STANDL. B, *S. appendiculata* K. SCHUM. 3, radial sections: A, *S. foetida* L. B, *S. oblonga* MAST. (All $\times 33$.)



1



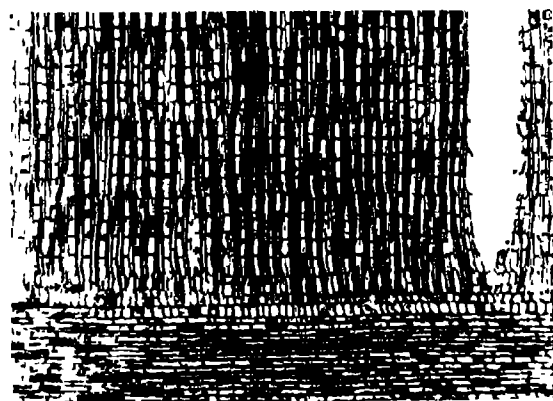
1



2

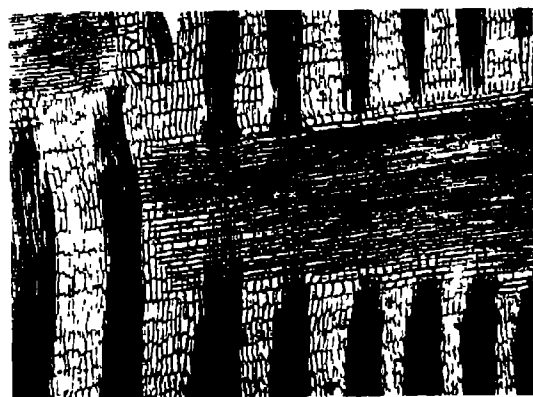


2



3

FIG 35 *Sterculia* A



3

FIG 36 *Sterculia* B

VIII—THE DEVELOPMENT AND MORPHOLOGY OF THE TEETH OF *ORNITHORHYNCHUS**

By H. L. H. H. GREEN, M.A., M.D.

Fellow of Sidney Sussex College and Lecturer in Anatomy in the University of Cambridge

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I—INTRODUCTION

Though tooth development in the mammals has been extensively studied, I had several objects in view when I decided to investigate the details of tooth development in *Ornithorhynchus*. The following are among the more important considerations which determined me to undertake this work.

1—*Early Stages in the Differentiation of the Dental Lamina and the Formation of the Enamel Organ*

The structure of the dental lamina and the stages in the early differentiation of the enamel organ have been carefully examined in representative groups by many observers, and by the beginning of this century the histological appearances of the dental lamina and its adnexa were well known. Yet the significance of some of the structures which are seen in the early stages of tooth development is still uncertain, and widely divergent

* This paper is substantially the same as a thesis which was recently accepted for the M.D. degree in the University of Cambridge.

(1933), in his book on vertebrate palaeontology, says on p. 256, "The anteater has no trace of teeth; in the duckbill there are a few irregularly shaped molar rudiments in the young which have been compared to some extent with those of the fossil Multituberculates."

Owing to the difficulty in obtaining material, knowledge of the dental system of *Ornithorhynchus* is very scanty. Our total knowledge of tooth development in the monotremes is based on the description of the conditions in four specimens of *Ornithorhynchus* (there are no dental structures to be observed in *Echidna*). POULTON (1889) originally described the structure of the enamel organs in a fairly late mammary foetus. The only measurement of this foetus that he gave was one of 83 mm. length "in the curled-up attitude in which it had been received". From his drawing WILSON and HILL estimated the snout-tail length (measured along the dorsal body curve) as about 250 mm. POULTON found in the upper jaw three large teeth with their principal cusps calcified, and posterior to them a further early tooth rudiment; in the lower jaw he also found three teeth, but the one corresponding to the most anterior one of the upper jaw was not recognized (the material was imperfect) though its presence was assumed as probable. He thus gave a dental formula of $\frac{4-4}{4-4}$ for *Ornithorhynchus*. WILSON and

HILL (1907) later described the dental lamina and enamel organs in two mammary foetuses, one of 250 mm. curved length ("Beta") which therefore closely resembled the specimen described by POULTON, and one much younger of 80 mm. curved length ("Delta"). They came to the conclusion that "representatives of five quasi-permanent teeth are developed in each jaw during the phases of tooth-development under consideration". Lastly, BROOM (1935) has briefly described the dental lamina in a foetal *Platypus* which he said was intermediate in size between specimens Delta and Beta but much nearer to the former. The specimen was very badly shrunk, but probably the snout-tail length when fresh was about 90 mm. BROOM demonstrated for the first time a disconnected anterior portion of the dental lamina of the lower jaw, in which were indications of three teeth; he showed that the probabilities were in favour of these representing two incisors and a canine. His determination of the dentition was $i \frac{0}{2} c \frac{0}{1} pm \frac{4}{4} m \frac{2}{2}$. I shall discuss these findings later in the light of my own results.

So far as I know no other observations on monotreme tooth development have been made. Further investigation into the dental system of such a primitive mammal is clearly to be desired.

The fully erupted teeth of the *Platypus* have been described by THOMAS (1889), STEWART (1892), and most recently by SIMPSON (1929). These observers agree as to the dental formula on eruption, it is $\frac{3-3}{3-3}$. The most anterior tooth of the upper jaw is very small, and the most posterior one in the lower jaw is considerably smaller than the two preceding teeth.

II—MATERIAL AND METHODS

Thanks to the kindness of Professor J. T. WILSON I have been fortunate enough to have at my disposal a fairly comprehensive series of stages of the developing *Platypus* for examination. The specimens were derived in part from Professor WILSON's private collection, in part from Professor J. P. HILL, and in part from the collection of the late Professor HARRISON in Sydney. The latter collection was presented by Mrs. HARRISON to the Department of Anatomy in the University of Sydney, and a number of the specimens included in it were lent to Professor J. T. WILSON in Cambridge and were made available for the purposes of the present investigation.

I should like to take this opportunity of thanking both Professor WILSON and Professor HILL, not only for giving me access to such a generous amount of material, but particularly for the interest they have shown in the work and for their valuable advice.

I am greatly indebted to Miss STEAD of the Anatomy Department of University College, London, for making a series of drawings of the models for reproduction.

Finally, my thanks are due to Mr. WALTER J. CALCOTT of the Anatomy Department of Cambridge University for his skilful assistance in preparing the sections and in doing the necessary photography.

Details of the foetuses examined in the course of this work are as follows [N.B. Snout-tail length is measured along the dorsal body curvature in each case]:

1—*Platypus* W (Wilson). 16.5 mm. "straight" length. Snout-tail length approximately 28 mm.

2—*Platypus* WW (Hill). Twin specimen of W.

3—*Platypus* X (Hill). Snout-tail 56 mm.

4—*Platypus* Delta (Wilson). Snout-tail 80 mm.

5—*Platypus* XXVIII B (Wilson). Snout-tail 122 mm.

6—*Platypus* H.N. (Harrison). From twin young found Namoi River, N.S.W. Snout-tail 140 mm.

7—*Platypus* H.J. (Harrison). From the nest, Namoi River, N.S.W. Snout-tail 170 mm.

8—*Platypus* H.P. (Harrison). Namoi River, N.S.W. Specimen rather dry, skin over trunk wrinkled. Snout-tail 200 mm.

9—*Platypus* H.Q. (Harrison). Namoi River, N.S.W. Skin of trunk also rather wrinkled. Snout-tail 225 mm.

10—*Platypus* Beta (Wilson). Snout-tail 250 mm.

11—*Platypus* H.X. (Harrison). Northcliffe Bank, N.S.W. Snout-tail 295 mm.

Taking the lengths of these foetuses as roughly proportional to their general stage of development, it will be seen that the series is reasonably complete; 28, 56, 80, 122, 140, 170, 200, 225, 250, 295 mm. respectively. Photographs of some of these specimens are shown in figs. 15–19, Plate 32.

Specimens W, WW, X, Delta, and Beta had already been serially sectioned. The remaining specimens I received as whole foetuses, and these were dealt with in the following manner.

In H.J. and H.P. the head was removed by transverse section to the body immediately proximal to the forelimbs. In XXVIII B and H.N. a similar transverse section was made, but in these cases the forelimbs were included in the head block. The blocks of XXVIII B, H.N. and H.P. were divided by a paramedian sagittal section, the smaller of the two blocks being cut into serial sagittal sections and the larger into a series transverse to the snout. In H.J. the whole head and neck region was cut transverse to the snout without any previous subdivision.

A block was removed from H.Q. which included the whole of the right side of the head and neck and the middle line as far back as the forelimbs, i.e. a left paramedian section was made. Again sections were taken transverse to the snout.

The head of H.X. was cut in a paramedian plane through the right nostril (fig. 18, Plate 32), and the smaller, right side of the head and neck was removed and sectioned sagittally.

In all cases the blocks were decalcified by means of 5% nitric acid in 90% alcohol. They were then embedded in celloidin and cut at thicknesses varying from 30 to 50 μ . The sections were all stained after cutting in haematoxylin and eosin except in the case of the smaller, left-hand block of H.P. which was stained in bulk in haematoxylin and eosin previous to sectioning. Surprisingly enough, the piece stained in bulk eventually gave the best series of sections from the point of view of both cutting and staining; the uniformity of the latter throughout the series and its differentiation in individual sections was considerably better than was obtained by the after-staining of the cut sections.

I have already given my reasons for undertaking the laborious task of making a series of reconstruction models. The models were made by the wax-plate method. From them one has the advantage of being able to visualize the more easily the relative sizes of the enamel organs, the pattern of the crowns of the teeth, and the spatial relations of the epithelial nodules both to the cusps and to the enamel organs.

III—DESCRIPTION OF THE DENTAL LAMINA, ENAMEL ORGANS AND TEETH IN THE VARIOUS STAGES

1—*Platypus* W

The dental lamina is a continuous structure in both jaws and is in a very early, undifferentiated stage. Its total length (in mm.) on each side is as follows:

Right side		Left side	
Upper	Lower	Upper	Lower
1.06	0.63	1.14	0.68

A model was made of the dental lamina of the left side and the associated mouth epithelium, using a magnification of 155 (figs. 20–22, Plates 32, 33). From this and from the graphic reconstruction shown in fig. 1 it will be seen that, at this stage, the lamina of the upper jaw extends forwards considerably beyond that of the lower jaw. Posteriorly the laminae terminate at much the same level, the upper lamina again extending slightly beyond the lower. In the upper jaw the lamina commences anteriorly immediately behind the egg tooth which is itself carried entirely upon the premaxillae as I have described in a previous publication (GREEN 1930).

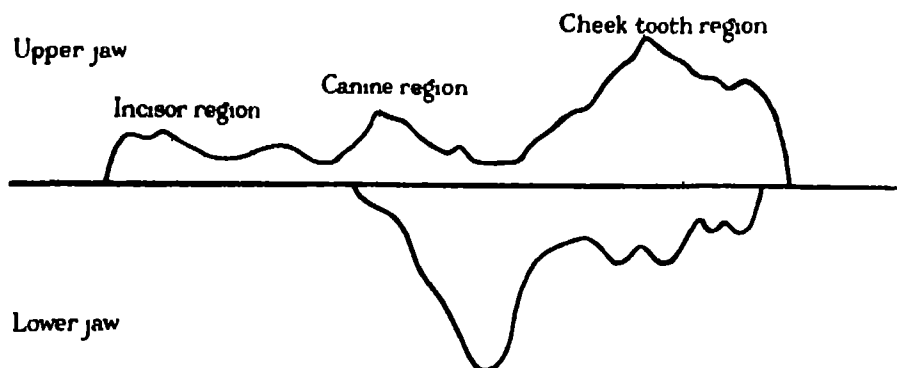


FIG. 1.—Graphic reconstruction of the dental laminae of specimen W. Notice the potential incisor region of the upper lamina; it is rapidly absorbed and is not found in later stages. $\times 77.5$.

In both jaws the dental lamina broadens considerably towards its posterior end. There is no differentiation of enamel organs, nor are any obvious localized swellings present which might indicate their future position. Nevertheless the surrounding mesoderm is markedly condensed around the laminae posteriorly (fig. 23, Plate 33).

A difference is to be observed in the vertical depth of the various parts of the laminae (fig. 1). Already the incisor and cheek tooth regions may be recognized with a shallower piece of the dental lamina connecting them; this shallow portion disappears almost immediately and leaves a diastema.

2—*Platypus* WW

This is the twin of the above specimen, though it is apparently more advanced in its development since the lengths of the dental laminae are (in mm.):

Right side		Left side	
Upper	Lower	Upper	Lower
1.72	1.21	1.82	1.31

The dental lamina of the lower jaw has thus grown at a relatively much greater rate than that of the upper jaw. The laminae of both jaws retain the same relations to each other posteriorly as before, i.e. the upper reaches back slightly beyond the lower (fig. 11), so that the relative excess of growth of the dental lamina at this stage has been

due to activity in the anterior portion of the lower jaw. Otherwise the dental lamina is in the same undifferentiated state as in specimen W.

The difference in the longitudinal extent of the laminae of the two sides is interesting; both W and WW show a predominance of growth on the left side.

3—*Platybus* X

Fig. 2 shows a graphic reconstruction of the dental laminae at this stage. It will be seen that a considerable amount of differentiation has occurred.

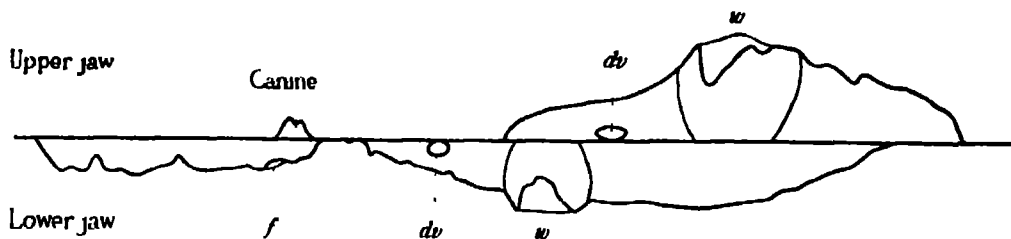


FIG. 2—Graphic reconstruction of the dental laminae and associated enamel organs of specimen X. $\times 37.5$.

The lamina of the upper jaw is now discontinuous; there is an isolated anterior portion measuring only 0.1 mm., then a gap of 0.68 mm., followed by the continuous posterior part of the lamina measuring 1.56 mm.

In the lower jaw there is only a short break of 0.17 mm. in the lamina which extends anteriorly for 0.97 mm. and posteriorly for 1.82 mm.

Though the posterior relations of the laminae remain the same as in the younger specimens, there is a striking difference anteriorly where the lower lamina now projects far in front of that of the upper jaw. This is partly due to the fact that, while the incisor region of the dental lamina is retained in the lower jaw, it has already disappeared in the upper jaw (compare W, WW and X in fig. 11).

The wax model (which was made at a magnification of 150) (figs. 24–27, Plates 34, 35) shows well the common labio-dental sheet anteriorly in the lower jaw, and the gradual separation of the dental lamina from the medial aspect of this sheet as it is traced in a backward direction.

I shall use the terminology adopted by WILSON and HILL (1907) to describe the teeth developed from the posterior part of the laminae, i.e. the letters “v”, “w”, “x”, “y” and “z”. These can all be looked upon as postcanine or “cheek” teeth. The separated anterior portion of the laminae which until now has only been mentioned by BROOM (1935) in the lower jaw of a single specimen, shows differentiations which, following BROOM’s terminology, I shall designate “a”, “b”, “c”, etc., since these, in all probability, are rudimentary representatives of the incisor region. In each case, a bar placed over a letter indicates that the particular tooth rudiment belongs to the lower jaw, while a bar under a letter means that the tooth referred to lies in the upper jaw. For example, “dv” represents the milk predecessor of tooth “v” of the upper jaw.

In this specimen there is no indication of enamel organ formation in the anterior part of the lamina except perhaps in the case of "*f*" (fig. 2), where a slight indentation of the free edge of the lamina occurs (this corresponds to BROOM's "*c*").

In the posterior portions of the laminae the nodules "*dv*" and "*dw*" are found indenting the deep aspect of the mouth epithelium lateral to the neck of the dental lamina. In neither jaw is there any convincing swelling of the lamina ("*kolbig*") such as WILSON and HILL described in relation to these nodules in specimen Delta. It is true that the lamina is swollen out at its free end, but no more markedly near these nodules than elsewhere. A thin shell of dentine is seen in "*dw*" on the surface of the mesodermal papilla (fig. 28, Plate 34), and it is interesting to observe how the mouth epithelium overlying the nodule is differentiated so that a "stratum intermedium" and a "stellate reticulum" can be recognized, the external enamel epithelium being here represented by a thick corneous layer. "*dv*" is clearly recognizable though no calcification has yet occurred in it.

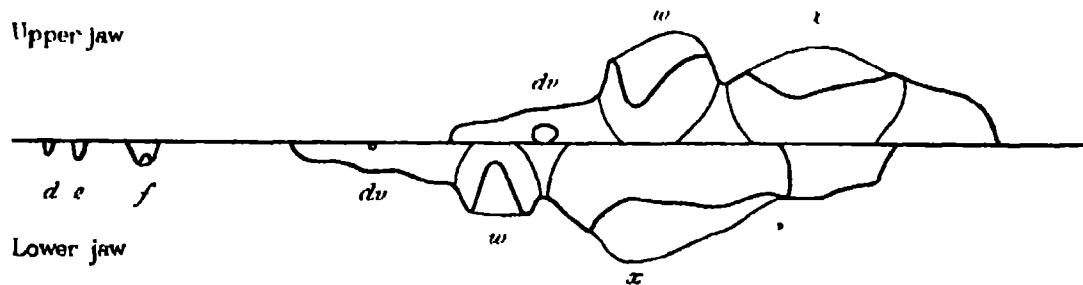


FIG. 3—Graphic reconstruction of the dental laminae and enamel organs of specimen Delta. $\times 27$.

Behind these nodules there is differentiated only one enamel organ in each jaw, "*w*". Both "*w*" and "*w*" are terminal enamel organs in BOLK's sense, and "*w*" shows the presence of a lateral enamel strand near its anterior portion.

The dental lamina behind "*w*" is very thick and swollen, with a capsule of condensed mesoderm, but it is not yet indented by a mesodermal papilla.

4—*Platypus* Delta

This is one of the two specimens described by WILSON and HILL in 1907. I have nothing to add to their detailed and accurate description of the condition of the developing dental lamina where it is seen as a continuous structure. I wish, however, to point to the presence of three small isolated portions of the dental lamina in the lower jaw which occur anterior to the main lamina. No separate anterior part of the upper lamina is seen in this specimen.

The complete picture of the dental lamina should therefore be as shown in fig. 3.

The main difference from the last stage is seen in the differentiation of the large enamel organ "*x*" in both jaws. The conical, pointed nature of the pulp cavity of "*w*"

compared with the elongated and comparatively shallow pulp cavity of "x" is obvious. As WILSON and HILL suggested, the probability is that "w" is a premolar and "x" a molar tooth. At this stage there is no indication of cusp differentiation in "x" except that the anterior part of the crown of the tooth is raised to a higher level than the posterior. "w" is now a parietal enamel organ in BOLK's sense; WILSON and HILL showed this clearly in their fig. 3 (1907), though the significance which BOLK attached to the position of an enamel organ relative to the dental lamina was not then recognized. As in specimen X there are indications of a lateral enamel strand in connexion with the enamel organ of "w": WILSON and HILL described indentations of the lateral side

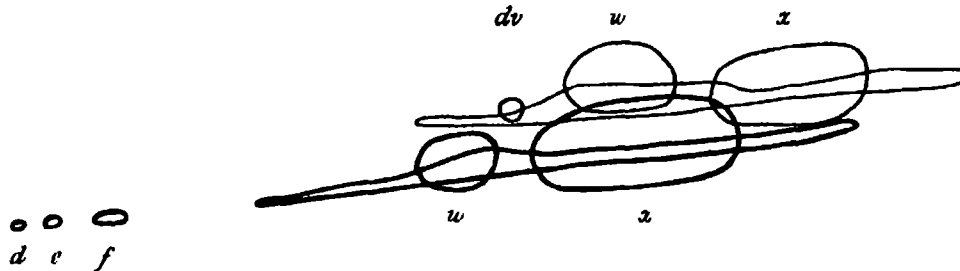


FIG. 4—Occlusion diagram of the dental laminae and the enamel organs of the right side of specimen Delta. Those of the lower jaw are represented by the thicker line. The horizontal line to the medial side of the laminae indicates the sagittal plane of the head. $\times 27$.

of the neck of the dental lamina, it is these that I interpret as representing pieces of a lateral enamel strand. The importance that these authors attached to these indentations as forerunners of the concentric epithelial bodies I shall discuss later.

The measurements of the laminae are as follows: lower jaw, 0.6 mm. in its anterior portion, then a gap of 0.63 mm., followed by the continuous posterior portion of 2.8 mm.; upper jaw, 2.6 mm.

The isolated piece of lamina "f" (fig. 3) is, as in the last specimen, slightly cup-shaped and has the appearance of a rudimentary enamel organ. "d" and "e" are undifferentiated downgrowths of the lamina.

A model of this stage is shown in figs. 29–32, Plates 35, 36. This was the first model that I made in the series, and I had not at that time suspected the presence of the anterior, disconnected part of the dental lamina, so that unfortunately it is not included in the model.

I have also made an occlusion diagram (fig. 4) to show how the dental lamina and the surfaces of the enamel organs of the two jaws are related to each other. The upper dental lamina and teeth are seen to lie as a whole lateral to those of the lower jaw, and the upper teeth lie posterior to the corresponding lower teeth.

5—*Platypus* XXVIII B

The graphic reconstruction in fig. 5 shows the condition of the dental lamina at this stage.

The measurements are as follows: upper lamina, an anterior piece of 0.1 mm., a gap of 1.47 mm. and the main lamina 3.09 mm.; lower lamina, anterior portion 0.9 mm., a gap of 0.7 mm., and the remainder of the lamina extending back for 3.2 mm.

Four separate pieces of dental lamina, "c", "d", "e" and "f", are seen in the anterior end of the lower lamina, of which "f" is again papillated; in addition, the dental papilla is now calcified and there is a well-defined stellate reticulum. It is interesting to notice that the isolated piece of the upper lamina which was absent in the last specimen is again present and occupies the same relative position as it did in specimen X.

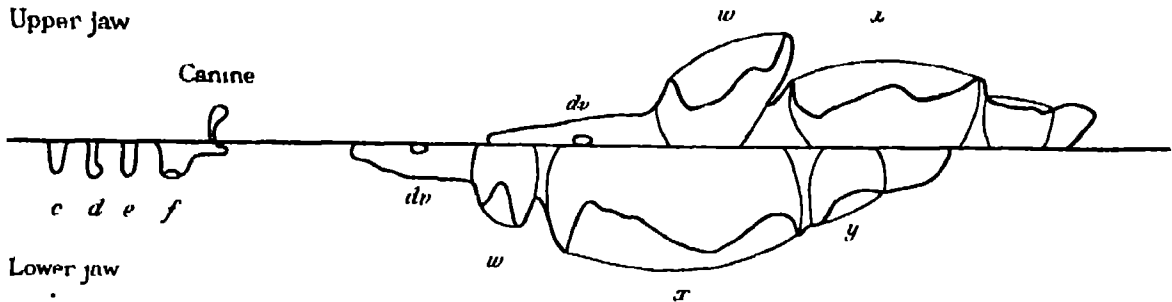


FIG. 5—Graphic reconstruction of the dental laminae and enamel organs of specimen XXVIII B. $\times 24.3$.

In the main lamina there has been a general backgrowth of the upper jaw relative to the lower so that the position of "*dv*" is now opposite the anterior cusp of "*x*"; in Delta it lay between "*w*" and "*x*". Similarly "*w*" has moved back in relation to "*x*" (fig. 11).

Since the last stage it will be noticed that the enamel organs of tooth "*y*" are now formed in both jaws. The drawings of the wax model (figs. 33–35, Plate 36) show these and other details more clearly than they can be described.

Immediately in front of "*dv*" there is a definite indication of an aborted tooth rudiment; this would correspond to the succeeding tooth "*v*", the presence of which WILSON and HILL surmised in Delta.

At this stage "*w*" is clearly a parietal enamel organ, since the free edge of the dental lamina is seen on its medial aspect; this is the case also with "*w*". Since in the earlier specimen X these enamel organs were terminal in position, it does not appear as though there were any fundamental difference in the position of an enamel organ relative to the free edge of the dental lamina as BOLK has claimed. Indeed, MARCUS (1931) comes to the same conclusion as a result of his investigations in various forms. It seems that all enamel organs arise originally at the free edge of the dental lamina and then become parietal in position, presumably as a result of the further growth of the free end of the lamina.

The enamel organs of "*w*" no longer show the presence of a lateral enamel strand; this was present in specimens X and Delta.

Both teeth "*w*" are well calcified.

Tooth "x" in both jaws shows the differentiation of two cusps, anterior and posterior; the anterior one is much the more differentiated, being higher and less rounded than the posterior. In the lower jaw "x" shows an irregular disturbance on the lateral side of the dental lamina, and towards the posterior end this resolves itself into a lateral enamel strand; similarly, in the case of "x" there is a well-marked lateral enamel strand posteriorly. At this stage "x" is a terminal organ.

There is no differentiation of the surface of the dental papilla of "y", it is still smoothly convex towards the ameloblastic layer of the enamel organ. There are indications in "y" of a lateral enamel strand. Both these enamel organs are terminal in position.

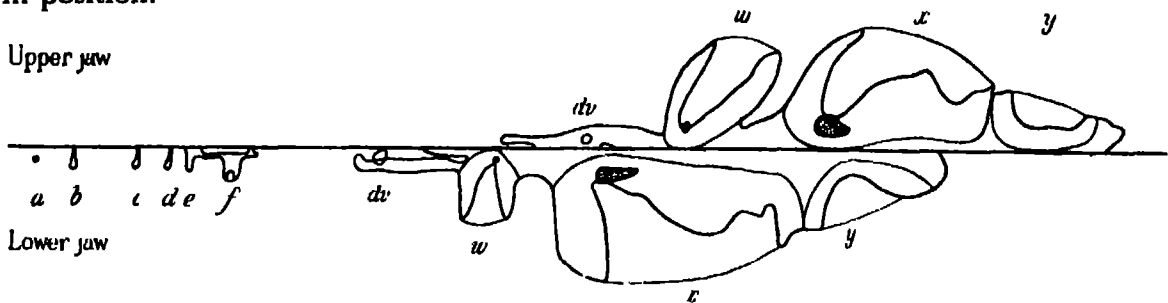


FIG. 6—Graphic reconstruction of the dental laminae and enamel organs of specimen H.N. The epithelial nodules appear in this specimen for the first time and are shown as stippled areas in the reconstruction: they are seen to lie close to the apices of the cusps. $\times 20.5$.

6—*Platyus* H.N.

A graphic reconstruction from the transverse series of sections is shown in fig. 6.

The upper lamina measures 4 mm.; the anterior part of the lower lamina is 1.5 mm. in length, then there is a gap of 0.63 mm., and the rest extends back for 3.75 mm.

The maximum development of the anterior part of the lower lamina is attained in this specimen. There are six separate downgrowths, "a", "b", "c", "d", "e" and "f". Of these, "e" shows a dark cap of mesodermal cells around its free end and gives every appearance of being a degenerating tooth; in "f" a minute calcified dentinal nodule is present; the remainder are simple downgrowths of the lamina, and in the case of "a" even the connexion with the lamina has been lost.

There is no trace of the separated anterior piece of the upper lamina.

In the lower jaw "dv" is represented by a densely staining dentinal nodule which is separated from the deep aspect of the mouth epithelium; the latter no longer shows any signs of disturbance. In the upper jaw "dv" has almost disappeared, though some bits of dentine scattered in a condensation of concentrically arranged cells reveal its presence.

The tooth rudiment "w" is calcified in both jaws and is clearly degenerating in the lower jaw (fig. 36, Plate 36). It has a single pointed cusp. The upper "w" shows a small epithelial body attached to the deep aspect of the external enamel epithelium and lying directly over the apex of the cusp. In the lower jaw "w" shows a rather more doubtful body in a similar position.

The tooth "x" shows a prominent anterior cusp which in the case of " \bar{x} " has a thin layer of dentine forming over the apex. A much lower and more rounded posterior cusp is differentiating. A large epithelial body is found overlying the anterior cusp in both jaws (figs. 37, 38, Plates 36, 37); it is embedded in the stellate reticulum, more deeply so in the case of the lower jaw than the upper. The enamel organ of "x" is parietal in position. In " \bar{x} " the external enamel epithelium forming the whole of the lateral wall of the enamel organ is ragged, and, as in specimen XXVIII B, a well-marked lateral enamel strand is found posteriorly (fig. 39, Plate 37). In addition to the anterior and incipient posterior cusps, " \bar{x} " possesses a prominent medial cingulum and " \bar{x} " a less prominent lateral cingulum. Already a few blood vessels are commencing to enter the stellate reticulum, particularly in the neighbourhood of the cusps, but they do not as yet penetrate far into the enamel organ.

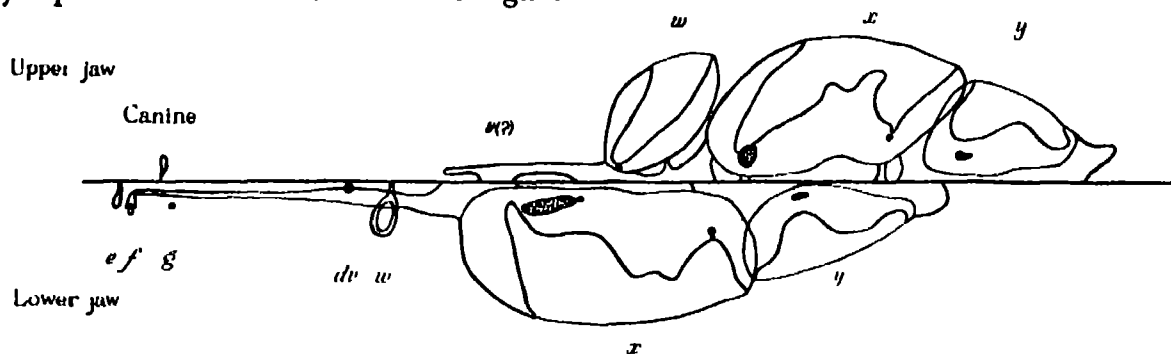


FIG. 7.—Graphic reconstruction of the dental laminae and enamel organs of specimen H.J. Epithelial nodules (stippled) " dx_1 " and " dy_1 " are found in both jaws, whereas " dx_2 " is only just differentiating. $\times 16.4$.

The enamel organ of " \bar{y} " shows the dental papilla to have a raised lateral margin, most marked anteriorly; this is the beginning of the future antero-lateral cusp. In the upper jaw " \bar{y} " shows but little differentiation; the papilla is more convex in front than behind. There is a lateral enamel strand in relation to the posterior part of the enamel organ of " \bar{y} ".

7—*Platypus* H.J.

Fig. 7 is a graphic reconstruction of the dental organs in this specimen.

Measurements of the laminae are as follows: upper jaw, 0.045 mm. anteriorly, a gap of 2.31 mm. and a posterior portion of 5.24 mm.; lower jaw, 0.045 mm., a gap of 0.06 mm. and then a continuous lamina extending for 6.49 mm.

It is noticeable that the dental lamina of the lower jaw is traceable in this specimen continuously from " f " backwards; it is true that it does not join the mouth epithelium until just in front of the enamel organ of " x ", nevertheless it is present in a region where in the previous specimens there has been a complete break.

The isolated piece of the upper lamina is present only on the right side. It is, as in specimens X and XXVIII B, situated opposite the anterior part of the maxilla, close to the posterior end of the premaxilla. It is swollen at its free end and in the centre of

the swollen part is a lighter area as though a stellate reticulum were trying to form (fig. 40, Plate 37). I consider that this represents the last persisting trace of an upper canine tooth.

In the lower jaw representatives of teeth "*e*" and "*f*" are present. The epithelial fragment "*e*" shows itself to be a degenerated tooth rudiment, the mesoderm around it is condensed and there appear to be a few specks of dentine developed. The tooth rudiment "*f*" has a cupped enamel organ with an obvious little dentinal nodule (fig. 41, Plate 37). From the persistent way in which the enamel organ of "*f*" is formed in all these specimens it would appear that it was a tooth of some importance; from its position in relation to the upper tooth (it is constantly situated immediately anterior to the latter), which I think is a canine, I suggest that "*f*" is the vestige of the lower canine.

Behind "*f*" there is an isolated rudiment "*g*" recognizable on both sides of the jaw (fig. 7). On the left side there is a definite dentinal nodule, on the right side no dentine is formed. It should be noticed that in each of the previous specimens there was a piece of dental lamina extending for a short distance behind "*f*" though no differentiation occurred in it. It is possible, as BROOM suggests (1935), that "*g*" represents a second canine. This would fit in with the condition known to occur in many Therapsida, whereas the alternative interpretation of "*g*" as an anterior premolar does not seem likely with such an extensive diastema behind it.

In the lower jaw "*dv*" is present on the left side as a simple, rounded, densely staining nodule of dentine (fig. 42, Plate 37); on the right side there is a nodule composed of dentine in its superficial part and of concentrically arranged mesodermal cells in its deeper part. In each case the nodule is placed deep to the mouth epithelium and causes no disturbance of the latter. There is no trace of "*dv*".

The upper dental lamina in the region where "*dv*" has previously been present widens out and becomes flattened or even somewhat cupped on its deep aspect for a distance of about 0.36 mm. This I take to be the enamel organ of "*v*" which is later aborted. It is to be remarked that in this situation the dental lamina retains its continuity with the mouth epithelium, while in front and behind "*v*" it is free.

The rudiment of tooth "*w*" is in an interesting condition. It is lying dissociated from the dental lamina and on its lateral aspect. It is composed of a thick capsule of faintly staining cells derived from the enamel organ, in the middle of which is a darkly staining mass of dentine (fig. 43, Plate 38). On the right side it seems to get a slight secondary connexion to the deep aspect of the mouth epithelium lateral to the dental lamina. Of the small epithelial body which was related to its cusp in H.N. there is no trace. Behind "*w*" for a short distance there are only scattered epithelial islets representing the dental lamina which is being absorbed. Indeed, it will be seen that the dental lamina shows signs of absorption everywhere except posteriorly, and no longer has any attachment to the mouth epithelium in many places. From the appearance in the upper jaw it would seem that the lamina retains its connexion with

the epithelium lining the mouth longest in those regions which lie opposite the cusps of the teeth.

The enamel organ " \underline{w} " occupies a position considerably lateral to the dental lamina, and it seems to acquire secondary attachments to the mouth epithelium still more laterally as described in the case of " \bar{w} ". There is dense calcification over the cusp of this tooth, which, towards the apex, shows signs of degeneration. The basal half of the tooth, however, has not yet acquired any dentine, and the enamel organ as a whole appears active and shows no sign of degeneration except for the apical dentine. It is in complete contrast to the corresponding tooth in the lower jaw at this stage, though it clearly shows a very early stage of degeneration of the type that has led to the condition of " \bar{w} ". A minute epithelial body appears to be present just lateral to the apex of the cusp on the left side; on the right side it is absent.

The tooth " \bar{x} " has a prominent and pointed antero-lateral cusp with a thin layer of dentine forming over it. Just within the external enamel epithelium and lying above and lateral to the apex of the cusp is a large epithelial body extending antero-posteriorly for 0.32 mm.; it is clearly a degenerate structure formed of a mass of cornified cells whose nuclei can be faintly seen containing many keratin granules; there is no differentiation of the stellate reticulum around it. A second small epithelial body of similar structure is found in the enamel organ just behind the first; similar detached fragments of an epithelial nodule are present in relation to " \underline{dy}_1 " of specimen Beta. Here and there the external enamel epithelium is broken up into such small islands that the stellate reticulum appears to be continuous with the mesoderm of the subcutaneous tissue. The postero-lateral cusp is now well defined and rises to a point in relation to which is a darkly staining area in the stellate reticulum, apparently continuous with the cusp of the tooth though not showing any ameloblastic structure. Calcification has not yet started in this cusp. The medial cingulum is developing and posteriorly rises into a rounded cusp.

The tooth " \underline{x} " has a pointed and calcified antero-medial cusp with an epithelial body, 0.2 mm. long, lying directly over it close to the external enamel epithelium. The structure of the body is similar to that described in the case of the corresponding nodule of the lower tooth. The postero-medial cusp is not calcified and shows, as in " \bar{x} ", a darkly staining patch of cells in the stellate reticulum closely related to the apex of the cusp (fig. 44, Plate 38). There is a rounded lateral cingulum. The enamel organ is parietal in position, and in its posterior part are seen the fragmentary remains of the lateral enamel strand noted in H.N.

An antero-lateral cusp has differentiated in " \bar{y} " but has not yet started to calcify; in relation to it there is an epithelial body which is deeply embedded in the enamel organ but lies rather nearer to the external enamel epithelium than to the apex of the cusp. A median longitudinal sulcus is differentiated in the posterior part of the crown of the tooth, but no posterior cusp has yet been formed. There is no lateral enamel strand in relation to this tooth.

Tooth "y". The large antero-medial cusp is not calcified; midway between the apex of this cusp and the external enamel epithelium there is an epithelial body in an early stage of differentiation; it extends for 0.2 mm. and seems as though it might represent a later stage of the condition seen over the posterior cusp of "x̄". A postero-medial cusp is becoming differentiated but is still rounded. The lateral cingulum is not well developed. A lateral enamel strand is developed posteriorly (fig. 45, Plate 38), and, as it is traced backwards, it is found to be continuous with the lateral wall of one of the indentations of the lateral side of the neck of the dental lamina to which WILSON and HILL attached so much importance (fig. 46, Plate 38).

8—*Platyptus* H.P.

In this specimen I have made models of teeth "x" and "y" in both jaws (figs. 47–58, Plates 39, 40). The surface of the dental papillae has become complex and cusp formation is well marked. The amount of the crowns of the teeth that has started to calcify is indicated in fig. 59, Plate 40. In each case I have included the epithelial bodies in the models to show their relations to the developing cusps.

The three tooth rudiments are still present in the anterior part of the lower jaw as in H.J. and require no more comment.

In the lower jaw, "w", though fully calcified, is not in such an advanced state of degeneration as in H.J.; there is a well-marked stellate reticulum. In fig. 60, Plate 40, the tip of the cusp of this tooth is cut transversely; if further degeneration occurred, together with the separation of the tip from the main cusp, a structure similar to an epithelial nodule might be produced. In the upper jaw "w" is also calcified, but at the base of the main cusp there is an uncalcified portion of the tooth which runs postero-laterally as a low ridge.

The lower tooth "x" possesses a large and rather blunted anterior cusp, on the posterior aspect of whose slope a smooth, rounded swelling is forming. There is a marked transverse waist to the crown of the tooth. A large postero-lateral cusp is present, it has a very pointed apex which curves medially to overhang the body of the tooth. The medial margin or cingulum has two well-differentiated cusps (figs. 47, 49, Plate 39). Dentine is forming over a considerable extent of the anterior and over the summit of the postero-lateral cusp (fig. 59, Plate 40).

There are two epithelial bodies in relation to "x̄", one being associated with each main cusp. Related to the antero-lateral cusp is the body " dx_1 " (using WILSON and HILL's annotation); it is immediately under the external enamel epithelium and occupies exactly the same relative position as it did in H.J. It is composed of corneous cells with a marked fibrillar structure, the fibrillae being arranged concentrically; the centre of the nodule has degenerated into a faintly staining core. The nodule " dx_2 " over the postero-lateral cusp is situated about half-way between the cusp and the surface of the enamel organ, and is still connected to the cusp by a strand of cells (fig. 61, Plate 40); its precursor was probably the dark patch of cells noticed over the

cuspid in H.J. It is a corneous structure with some condensation of the cells of the stellate reticulum around it. It is interesting to observe that the dentine forming the apex of the postero-lateral cusp is cellular and shows signs of degeneration (fig. 61, Plate 40). It appears possible, especially in view of its origin in H.J., that " dx_2 " may represent the degenerated apical portion of what was originally a much more pointed cusp.

Tooth " x ". As seen from the model the antero-medial cusp is prominent. There is a marked waist to the tooth in the form of a deep and narrow transverse fissure which separates the antero-medial from the postero-medial cusp. The latter is well differentiated but has not attained the size of the anterior one. The lateral margin of the tooth is scarcely raised, though there is some indication of two small tubercles forming at its anterior end. The antero-medial cusp is calcifying, an area of dentine being formed over the apex and to some extent on the medial surface. Dentine is just beginning to form on the medial aspect of the apex of the postero-medial cusp.

There are three epithelial bodies associated with this tooth, one over each main cusp and one unrelated to the cusps. The nodule " dx_1 " (fig. 62, Plate 41) is an ovoid body elongated in the longitudinal axis of the tooth; it is spread out immediately under the external enamel epithelium and lies lateral to and somewhat behind the antero-medial cusp. It is not connected to the cusp and it is not encapsulated. It is composed of a mass of horny epithelial cells with many collections of keratin granules apparent. The second nodule, " dx_2 " (fig. 63, Plate 41), is just lateral to and above the tip of the postero-medial cusp, embedded deeply in the stellate reticulum. The core of horny cells is encapsulated by an aggregation of cells of the reticulum. It is not connected to the cusp at this stage, but its precursor was seen to be closely associated with the cusp in H.J. The third nodule, " dx_3 " (fig. 64, Plate 41), seems to be an adventitious epithelial body, since no similar structure has been seen in any other specimen in this situation. It appears as a bud from the deep aspect of the mouth epithelium which separates itself from the latter and forms a typical epithelial nodule between the epithelium of the mouth and the surface of the enamel organ. There is a core of horny cells more or less concentrically arranged and surrounded by a deeply staining capsule of undifferentiated epithelial cells: a structure, in fact, remarkably like what one would expect to find at a rather earlier stage in the formation of " dx_2 ". The nodule " dx_3 " is peculiar, not only in having no relationship to the developing cusps, but also by being considerably external to the whole enamel organ.

The lower tooth " y " has a pointed antero-lateral cusp whose apex bends medially. There is a constriction in the middle of the tooth, and a postero-lateral cusp is becoming prominent though it is still rather short and rounded. The medial cingulum is undifferentiated. Only the tip of the antero-lateral cusp is calcified. There is a single epithelial body, " dy_1 ", just medial to and above the antero-lateral cusp. It lies on the deep aspect of the external enamel epithelium and bulges it outwards, thus contrasting with the position it occupied in H.J. It is formed of a condensation of cells of the stellate reticulum with some keratinized cells in the middle. A dark band of cells

indicates its original connexion with the cusp. A strand running out into the reticulum from the apex of the postero-lateral cusp indicates the position in which a second epithelial body ("dy₂") will be formed.

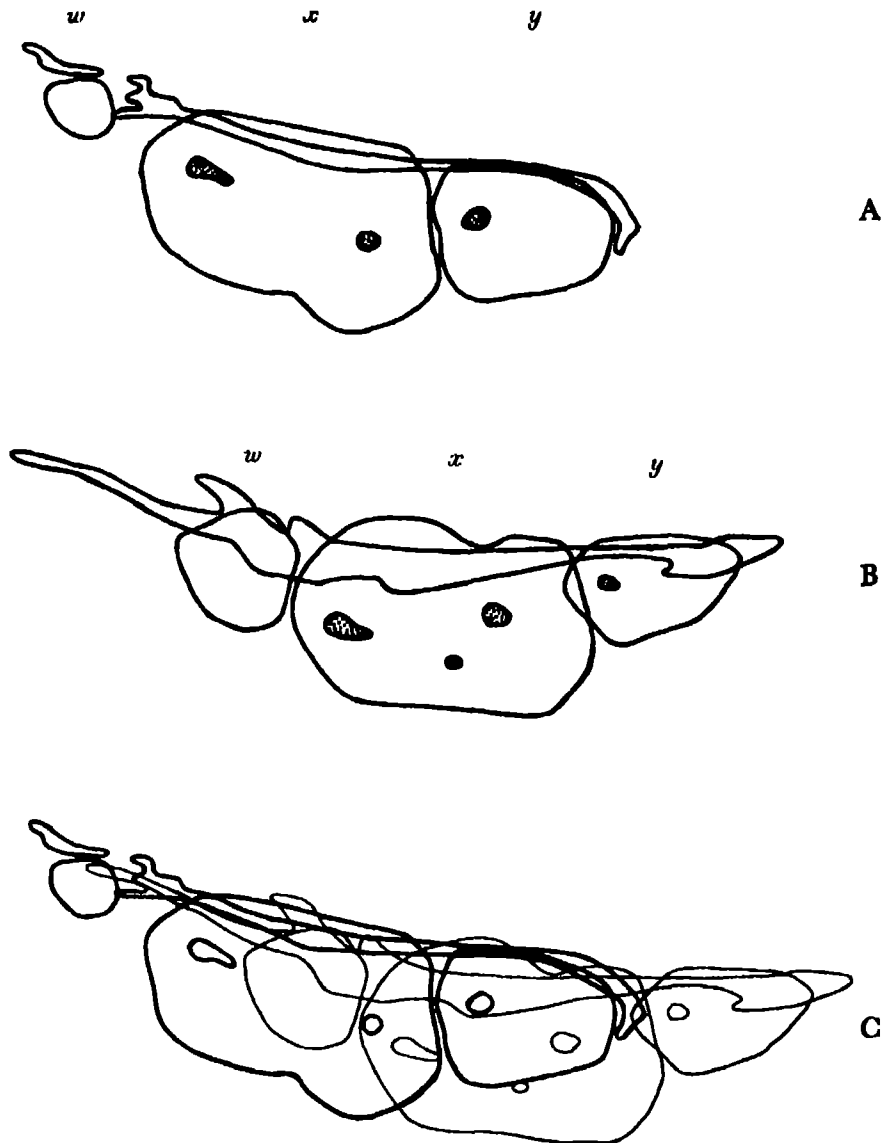


FIG. 8—Graphic reconstruction of the dental laminae, enamel organs and associated epithelial bodies of the left side of specimen H.P. in surface view. A, lower jaw; B, upper jaw; C, occlusion diagram. In A and B the epithelial nodules are stippled. $\times 16.65$.

The upper tooth "y" possesses a well-marked antero-medial cusp with a deep transverse fissure behind it. This fissure indents the medial margin of the tooth but does not extend to the lateral cingulum. The latter is smooth and shows no sign of differentiation into cusps. Behind the fissure a low, rounded postero-medial cusp is commencing to form. No calcification has yet begun. There is an epithelial body, "dy₁", in the stellate reticulum, lying behind and lateral to the antero-medial cusp

and some distance from it. Its relationship to the cusp is perhaps indicated by a stalk which connects the anterior end of the body with the stratum intermedium over the apex of the cusp. This nodule does not show a concentric arrangement of cells and appears to be no more than an aggregation of the cells of the stellate reticulum, many of which in the centre have undergone cornification.

Fig. 8 shows an occlusion diagram of the dental laminae and the enamel organs of this specimen. The condition in each jaw is also shown separately. It will be noticed that the epithelial bodies of the upper teeth alternate with and are placed lateral to those of the lower teeth; this is what might be expected if they represented the apices of the original main cusps of the teeth.

9—*Platypus* H.Q.

The general condition of the dental laminae and of the enamel organs is so similar to that of the last specimen, H.P., that I will confine myself to describing any important differences.

The upper "*w*" seems to reach its maximum differentiation at this stage. It is a definite tritubercular tooth with all three cusps calcified. There is the main large antero-medial cusp which has been seen in all the preceding specimens; towards the posterior part of the base of this cusp lies a smaller but pointed antero-lateral cusp; behind the latter is a tall and needle-like postero-lateral cusp (fig. 9). These two lateral cusps are lost in later stages, though it will be seen in H.X. that there is some indication of the position they occupied in the postero-lateral expansion at the base of the main cusp.

In both upper and lower teeth "*x*" and "*y*" a dark strand of cells is to be seen running from the stratum intermedium covering the cingulum outwards for a short distance towards the surface of the enamel organ. This strand is related to that part of the cingulum which lies opposite the anterior part of the main posterior cusp, or just in front of that. It is possible that these strands may indicate the position of what were once more prominent cusps.

The enamel organs of tooth "*z*" are indicated in both jaws as thickenings of the posterior end of the dental lamina. These thickenings have paler centres, where the future stellate reticulum is commencing to develop.

The epithelial bodies are almost identical in both structure and position with those described in H.P. The nodule " \overline{dx}_1 " (fig. 65, Plate 41) shows the lamellar structure of the concentrically arranged fibrils with a central core of large degenerated cells; it is very similar to the corresponding body in H.P. When compared with the last specimen, the chief differences found in the epithelial bodies are the following. The lower " dx_2 " (fig. 66, Plate 42) and the upper " dy_1 " are now fully cornified and there is no longer any capsule to be distinguished separating them from the stellate reticulum. The epithelial body " \overline{dx}_2 " is found to lie just under the external enamel epithelium instead of being deeply embedded as it was in H.P. There is no trace of " \overline{dx}_3 ". There is a

second nodule now formed in relation to tooth "y"; this nodule, "dy", is seen as a condensation in the stellate reticulum just over the postero-medial cusp and closely related to it; it is much nearer the cusp than to the surface of the enamel organ. Some cornification is starting in its centre.

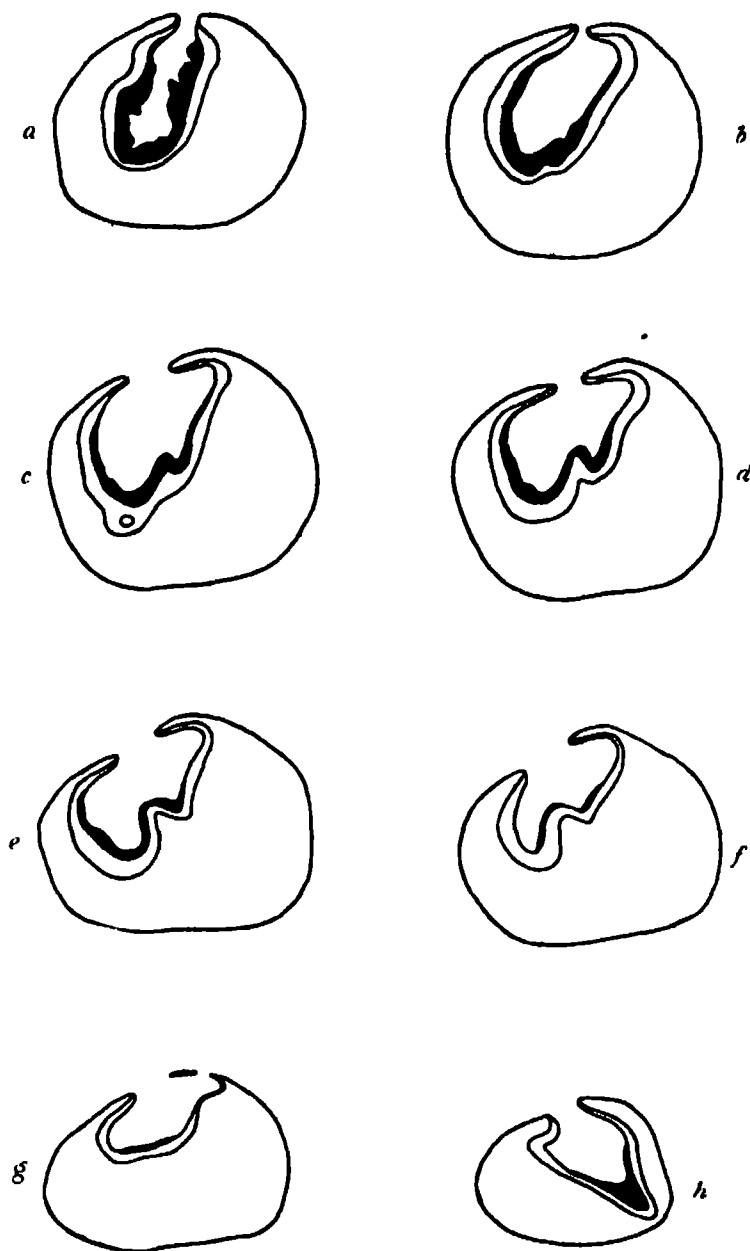


FIG. 9.—Specimen H.Q. A series of drawings of transverse sections through the enamel organ of the upper right tooth "w". The sections run antero-posteriorly from *a* to *h*. The heavy lines indicate the amount of dentine that is formed. In *c*, *d*, *e* and *f*, the antero-lateral cusp is seen springing from the main antero-medial cusp. In *h* the needle-like postero-lateral cusp is present. $\times 30$.

10—*Platybus* Beta

This is the older of the two specimens whose dental organs were originally described by WILSON and HILL. I have made models of teeth "x" and "y" in both jaws, primarily to show the relationship of the epithelial nodules to the cusps (figs. 67-78, Plates 42, 43). In fig. 79, Plate 43, I have also indicated the amount of dentine formation which has occurred in the crowns of the teeth.

On comparing these teeth with those of H.P. the extraordinary increase in the complexity of the crowns is apparent. Moreover, the increase in the size of the teeth is astonishing as the following table shows; Beta is only 50 mm. longer than H.P. in its curved snout-tail length. The actual lengths (in mm.) of the crowns of the teeth are given:

	"x"	"y"	"x"	"y"
H.P.	2	1.2	2.2	1.1
Beta	4	3	3.5	2.8

It will be noticed from a comparison of the models that a large proportion of this increase is due to the growth and differentiation of the posterior parts of the enamel organs.

Calcification has started in the main cusps of all these teeth; it is most marked in the anterior cusp of "x" and least marked in the posterior cusp of "y", so that calcification is proceeding from before backwards. It is also more extensive in the lower jaw than in the upper, since, whereas the cingulum of the upper teeth has not yet started to calcify, four cusps on the cingulum of "x" and one on that of "y" have already acquired a dentinal covering (fig. 79, Plate 43). On the whole, the most prominent parts of the tooth calcify first, but it will be noticed that the most anterior of the cusps on the cingulum both in tooth "x" and "y" are calcified though they lie below the level of many of the cusps which have not yet formed any dentine; this may have some phylogenetic significance. The prominence of the cusps on the cingulum in the lower teeth is a striking feature.

The epithelial nodules in this specimen require some attention. Their constant relationship to the main cusps of the teeth is again clear. Since WILSON and HILL described the nodules in this specimen in great detail, and since, without having the assistance of any nearly related stages of development, they drew inferences of considerable importance from the conditions they found, I should particularly like to draw attention to one or two points.

The nodules " \overline{dx}_1 " and " \overline{dx}_2 " do not differ in any essentials from the corresponding bodies in the other specimens except that " \overline{dx}_2 " is now found immediately under the external enamel epithelium instead of being deeply situated as in H.Q. The lower " \overline{dy}_1 " is much larger than it was in H.P. and H.Q. but is otherwise similar (this is the nodule called " \overline{dy}_2 " by WILSON and HILL). WILSON and HILL's nodule " \overline{dy}_1 ", which they thought might be related to tooth "x", is very small and lies immediately under the epithelium of the mouth and seems to me to be comparable with " \overline{dx}_2 " of specimen

H.P. It is not in series with the other nodules; it is not closely related to the enamel organ; it is not found on the other side of the jaw; it is not found in the other specimens; therefore I consider it to be an adventitious structure. The lower " dy_2 " (WILSON and HILL's " \overline{dy}_3 ") is now formed as a small nodule closely related to the postero-lateral cusp; it was only indicated as a darker area in the stellate reticulum in H.P. and H.Q.

The two nodules " dx_1 " and " dx_2 " are found as before; the latter has become strongly keratinized and its centre has degenerated to form a core, so that its structure is very similar to that of " \overline{dx}_1 " of H.Q. The upper " dy_1 " has increased in size and has a few small accessory epithelial bodies lying close to it on its medial side.

Immediately posterior to " dy_1 " lies the undoubted dentinal nodule which WILSON and HILL designated " dy_2 ". This nodule is so unlike any of the other epithelial bodies, that, to say that it is in series with them, as WILSON and HILL claimed, is only justifiable from the point of view of its position. Even its position is in fact different in that it is embedded in the external surface of the enamel organ and is recessed into it from outside, whereas all the epithelial nodules are, from their earliest appearance, inside the enamel organ. In structure this nodule shows all the characters of a complete enamel organ; there is not only the ring of dentine and the pulp, but a fully developed ameloblast layer of cells surrounded by a stellate reticulum. Such a structure is not approached by any of the epithelial bodies at any stage of their development. WILSON and HILL stated that on the opposite side this nodule was represented by a "typical concentric epithelial nodule"; with this I disagree, as the only structure I can find which occupies approximately the same position on the other side of the jaw is a split off piece from the deep aspect of the mouth epithelium and it lies wholly separated from the enamel organ, close to the epithelium lining the mouth cavity. It is not comparable in position or structure with a typical nodule; it is far more like the adventitious body described as " dx_3 " in H.P. A structure of such size, differentiation, and importance as this dentinal nodule should be represented in some way in other stages of development of the *Platypus*. I find nothing to correspond to it either in the earlier specimens or in H.X. The only possible antecedent is an epithelial body in H.P. which lies in a comparable situation and which is attached to the deep aspect of the mouth epithelium (fig. 80, Plate 44); it only extends over two sections and is unrepresented in H.Q. or H.X. I am, therefore, forced to the conclusion that this dentinal body is adventitious and is peculiar to the one side of the jaw in this particular specimen. In further support of this is the fact that it is additional to the constant epithelial body " dy_1 ", it does not replace the latter as might be expected if it were a further differentiation of such a body.

The nodule " dy_2 " is present over the postero-medial cusp.

11—*Platypus* H.X.

This specimen measured 295 mm. in length. According to BURRELL (1927), a foetus of this size would have been nearly ready to leave the nest and would be approximately

6 weeks old. The teeth at this stage have almost attained the size of those figured by SIMPSON (1929) in his specimen A which was aged $8\frac{1}{2}$ weeks.

The teeth of the *Platypus* would therefore appear to erupt between the 6th and the 8th week.

The actual size of the teeth has not greatly changed from that found in Beta; "x" remains about the same, though "y" has grown considerably in both jaws. Nevertheless, calcification has increased to a marked extent. In the lower jaw of this specimen, "x" is fully calcified, and only the lowest part of the basin and the posterior margin of "y" are devoid of dentine; in the upper jaw, "x" is devoid of dentine over a small area at the bottom of the basin, and "y" has none covering most of its posterior half except for the postero-medial cusp. Comparing this with fig. 79, Plate 43, which shows the amount of dentine formation in Beta, the increase in the amount of calcification is obvious.

As in all the preceding specimens, tooth development is more advanced in the lower jaw than in the upper.

There is developed at this stage a thick covering of enamel over the cusps of the teeth (fig. 81, Plate 44). No enamel was formed in Beta. It will be seen from the drawings of the models of this specimen (figs. 82-87, Plates 45, 46) that, roughly speaking, enamel has formed over those areas which in Beta were covered with dentine.

As will be discussed later, this enamel formation seems to depend on the intimate association of the capillaries which grow into the enamel organ with the stratum intermedium. In Beta the enamel organs are vascular, but the capillaries (which are most numerous in the neighbourhood of the cusps) have not yet penetrated as far as the stratum intermedium. In H.X., not only have the enamel organs become far more vascular, but the vessels in the areas where enamel is being formed are only separated from the ameloblasts by the thin stratum intermedium. The great vascularity of the enamel organs is well seen in fig. 81, Plate 44.

Perhaps the most striking thing about this specimen is the variety of evidence which it offers to show the extraordinary state of degeneracy of the teeth. The following features are particularly noticeable in this connexion:

- 1—The irregularity of the surface of the deposited enamel (figs. 93, 97, Plates 47, 48).
- 2—The irregularity and folding of the ameloblast layer, especially over the cusps.
- 3—Scattered islets of enamel are seen over the apices of the cusps, embedded in the stellate reticulum, and running out towards the epithelial bodies (fig. 94, Plate 48).
- 4—Ameloblastic strands are continued from the apices of the cusps for some distance towards the surface of the enamel organ. These strands appear late in development; they are foreshadowed in H.Q. but are only apparent as a double row of ameloblasts in Beta. WILSON and HILL (1907) suggested that these epithelial cylinders or strands represent portions of the cusps which have undergone ontogenetic reduction. I think there can be no doubt that this is the correct interpretation.

I have made two sets of wax models to show the state of the dentition in H.X. In the one set (figs. 82-87, Plates 45, 46) I have modelled the crowns of the teeth and indicated the extent of enamel formation; the other set (figs. 88-91, Plates 46, 47) represents the whole enamel organs with their surfaces cut away here and there to show the relations of the epithelial bodies to them.

The following account gives some details of the dentition at this late stage of development.

The tooth rudiment " \bar{y} ", which was present in Beta, is no longer developed. On the other hand, differentiation has proceeded posteriorly and " \bar{z} ", which in Beta was present as a small papillated enamel organ, has now differentiated into a tritubercular tooth, though, as yet, calcification has not commenced in it. In the upper jaw, where in Beta there was a thickened posterior end to the dental lamina without any enamel organ, there is now an indication of a localized swelling with formation of a stellate reticulum though there is still no actual papillation. Thus the anlage of an upper " z " is apparent in this specimen; it would seem that it never proceeds to the stage of actual tooth formation or, at any rate, it never reaches the stage of eruption.

In the lower jaw " w " is a degenerate nodule of dentine with a cap of enamel covering it. The remains of the stellate reticulum are still visible (fig. 92, Plate 47).

The lower tooth " x " shows two very prominent cusps, the summit of the anterior one being blunt and wide transversely, while the postero-lateral one has a pointed apex. Both have a relatively thick enamel covering. The medial cingulum and the medial part of the posterior cingulum have developed five small cusps all of which are surmounted by enamel. The ameloblast layer is irregular and is folded into "humps" over the anterior and the postero-lateral cusps. The whole crown is calcified, the dentine being thickest over the cusps.

As before, there are two epithelial bodies in relation to the two main cusps. The nodule " \overline{dx}_1 " is in a very degenerate condition with a thin capsule of concentrically arranged fibres and a large core of shapeless cells (fig. 93, Plate 47). The lower " dx_2 " is a small keratinized body; between it and the postero-lateral cusp are a few small nodules of enamel in the stellate reticulum; these can just be recognized in fig. 81, Plate 44.

The lower tooth " y ". The floor of the central basin and the posterior cingulum of this tooth are uncalcified, the remainder of the crown being covered with dentine. There are three enamel-covered cusps, the two main lateral ones and the most anterior cusp of the medial cingulum. From both lateral cusps transverse ridges run across to the medial border of the tooth, the posterior ridge being the more prominent. The postero-lateral cusp is very low and rounded with no apical portion; there is, however, a large ameloblastic strand continued up towards the surface of the enamel organ in the position of the aborted apex of this cusp.

The two epithelial bodies usually associated with this tooth are present. The nodule " \overline{dy}_1 " has a degenerating centre and bulges out the external enamel epithelium to a

marked degree (figs. 81, 88, Plates 44, 46). Odd islets of enamel are found just deep to " \overline{dy}_1 " and some distance from the tip of the antero-lateral cusp (fig. 94, Plate 48). The second nodule, " \overline{dy}_2 ", is close to but not in contact with the external enamel epithelium. It is a typical horny body with no capsule separating it from the stellate reticulum.

In addition to these two bodies which appear to be constantly present, there is a nodule connected with the apex of the most anterior cusp on the medial cingulum which is indistinguishable from one of the epithelial bodies in their early stage of development (fig. 95, Plate 48). There are also two similar small epithelial nodules connected with the apex of the most posterior cusp of the medial cingulum. These extra nodules lie rather nearer to the cusp than to the external enamel epithelium and are connected in each case to the cusp by a strand of cells. They are exactly comparable with the early developmental appearance of epithelial bodies described in H.J. and H.P.

The lower tooth "z". At this stage "z" has not started to calcify. It has a large antero-lateral cusp, a smaller postero-lateral one, and a rounded cusp on the medial margin of the tooth. It is therefore of tritubercular nature at this stage. The fact that there are two prominent cusps developing on the lateral side of the crown indicates that this tooth is developing the same essential cusp pattern as the preceding cheek teeth.

Vascularization of the enamel organ has commenced and the vessels are directed in towards the apical region of the antero-lateral cusp.

In association with the antero-lateral cusp is a large epithelial body (fig. 90, Plate 47) which lies just deep to the external enamel epithelium and is connected to the apex of the cusp by a condensed strand of cells. This nodule, " \overline{dz} ", is of the typical cornified cell type.

The upper tooth "w". This tooth is fully calcified though the dentine is very degenerate and it is covered over its whole crown with a fairly thick layer of enamel. Only the one, medial, cusp is present, but the crown of the tooth expands laterally and to some extent posteriorly, thus indicating the position of the two lateral cusps found in H.Q.

An epithelial body " \overline{dw} ", composed of concentrically arranged horny cells with a degenerating core, is developed immediately over the tip of the single prominent cusp (fig. 96, Plate 48).

The upper tooth "x". The whole crown of the tooth is covered with dentine except for a small area in the floor of the central basin. The two medial cusps are very prominent and have a thick layer of enamel over them. There are six small enamel-tipped cusps developed on the lateral cingulum (fig. 85, Plate 45).

The nodules " \overline{dx}_1 " and " \overline{dx}_2 " both bulge out the surface of the enamel organ and are of the typical concentric arrangement with a central degenerating core.

Two small collections of epithelial cells are plastered on to the superficial surface

of the enamel organ medial to the apex of the antero-medial cusp. They are connected to the deep surface of the mouth epithelium and show no particular differentiation. I consider them to be remains of the dental lamina in this region. They are not comparable with the typical epithelial bodies.

The upper tooth "*y*". The posterior half of this tooth is uncalcified except for the postero-medial cusp. There are the two main medial cusps, each with an enamel covering. Two small cusps at the anterior end of the lateral cingulum have also enamel caps. The ameloblast layer is irregular and folded over the two medial cusps, and the tips of these cusps are clearly degenerate (fig. 97, Plate 48).

The epithelial bodies "*dy₁*" and "*dy₂*" are present over their respective cusps. The nodule "*dy₁*" is a concentric body with no obvious core; it lies over but slightly lateral to the tip of the antero-medial cusp and bulges out the surface of the enamel organ. The second nodule, "*dy₂*", is just deep to the external enamel epithelium and does not bulge it out; and it is formed of concentrically arranged keratinized cells, and a strand from the tip of the postero-medial cusp runs towards it.

There is no trace whatever of the dentinal nodule described by WILSON and HILL as "*dy₂*".

IV—DENTITION OF *ORNITHORHYNCHUS*

1—*Erupted Teeth*

It is well known that the erupted teeth of *Ornithorhynchus* number three on each side of both jaws. Using the terminology adopted in the description of the development of the dental lamina, these are $\frac{w, x, y}{x, y, z}$.

As I have already pointed out, since the teeth are not erupted in specimen H.X. and are erupted in SIMPSON's specimen A, it would appear that the time of eruption is between the 6th and the 8th week after hatching.

"*w*" is a very small tooth. It has a single, tall, sharp cusp. SIMPSON (1929) says on p. 3: "The figure and model of Stewart's specimen, on the contrary, show it as a minute elongate tooth with a flattened crown bearing about seven indefinite, low, rounded cuspules." This is not so. STEWART (1892) labels the structure which corresponds to "*w*" in his picture as a "soft papilliform structure in front of true teeth of the right side of the upper jaw, the first or most anterior tooth having been shed". It is probable that "*w*" is the last premolar tooth.

On account of the complication of their crown pattern, their relative size, and their position in the jaws, teeth "*x*" and "*y*" are certainly molariform in character.

The dental formula of the fully erupted teeth is therefore $i \frac{0}{0} c \frac{0}{0} pm \frac{1}{0} m \frac{2}{3}$.

2—*Developing Teeth*

Examination of the differentiation of the dental lamina in various foetal specimens has led to the following conclusions.

POULTON (1889) described four developing teeth in the upper jaw and, while only actually observing three in the lower jaw because his material was defective, he surmised the presence of another one corresponding to the most anterior of the upper ones. These teeth were $\frac{w, x, y, z}{w, x, y, z}$.

It is interesting to notice that POULTON stated on p. 23: "I think, however, that it is very probable that the rudiments of teeth may be found anteriorly at a much earlier stage, when the bill is less developed. . . ."

WILSON and HILL (1907) from the examination of their two specimens found evidence of another developing tooth just anterior to "w", giving a formula of $\frac{v, w, x, y, z}{v, w, x, y, z}$. They also described a milk predecessor to the most anterior of these teeth. The teeth were all situated far back in the jaws and were clearly of post-canine nature.

Recently BROOM (1935) has reported on the dental lamina of a very young foetus and has described an anterior portion of the dental lamina in the lower jaw which was completely separated from the main posterior part, and which gave evidence of the presence of three teeth which he called "a", "b" and "c". "c" possessed a definite enamel organ, and BROOM suggested that this was a canine tooth, and that "a" and "b" were two incisors. He also surmised the presence of a tooth "u" in each jaw just in front of "v". As a result, he gives the dental formula for *Ornithorhynchus* as follows:

$$i \frac{0}{2} c \frac{0}{1} pm \frac{4}{4} m \frac{2}{2}.$$

This means that BROOM looks upon "x" as the last premolar tooth, despite its position, form and general complexity. The reason for this assumption is that he finds "indications of there having been some rudiments of a *dw* and *dx*." He describes an irregular gap on the outer side of the necks of the enamel organs of "w" and "x", "as if a calcified tooth like *dv* had been removed and nothing put in its place. It looks as if Nature had thought of putting a little rudimentary tooth here, and had then thought it not worth while." I do not feel that it is justifiable on such evidence to postulate the presence of the rudiments of milk teeth. Certainly no calcified nodules appear in these situations in later stages of development. Also, the same "irregular gaps" are seen on the outer side of "y", in which case "y" would also have to be considered a premolar tooth. I think that these gaps correspond to BOLK's "enamel niche" and are more or less completely bounded laterally by his "lateral enamel strand".

In the series of specimens I have described, the differentiation of the anterior part of the dental lamina of the lower jaw can be followed until it appears to be most highly differentiated in H.N., where rudiments "a", "b", "c", "d", "e" and "f" are present. My "d", "e" and "f" correspond to BROOM's "a", "b" and "c".

In addition to this, in specimens X, XXVIII B and H.J. there is an isolated piece of dental lamina in the upper jaw which lies opposite to and immediately behind "f"

of the lower jaw. Its relation to the premaxilla and to the maxilla shows it to be almost certainly the upper canine. This would substantiate BROOM's view that the corresponding tooth of the lower jaw, i.e. "f", is the lower canine.

In confirmation of the suggestion that "f" was originally a tooth of considerable importance is the fact that in all my specimens in which it is present I find it represented by a papillated enamel organ, while in specimens XXVIII B, H.N. and H.J. an actual dental nodule is formed.

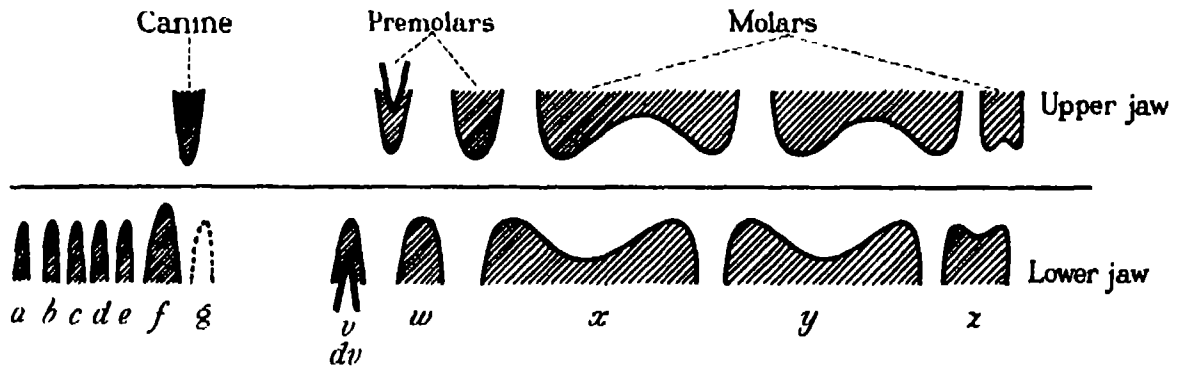


FIG. 10—An anachronistic diagram to show the ideal dentition of *Ornithorhynchus*. The absence of incisors in the upper jaw and the presence of a single replacing tooth in each jaw should be noticed.

The continuation of the dental lamina of the lower jaw backwards for a short distance behind "f", which BROOM described, is constantly present. Its continuity is broken in H.J., where this part of the lamina is represented by a small separated epithelial nodule which may be calcified. Whether this may be a second canine as BROOM tentatively suggests, or whether it is the most anterior premolar, I am unable to say.

Though the dental lamina of both jaws is always continued for some distance in front of "v", I have been unable to find any evidence of a localized thickening which might indicate a tooth "u" in any of the specimens. The mere presence of a continuous stretch of undifferentiated dental lamina is hardly sufficient to justify BROOM's statement that it probably represents an anterior tooth.

The posterior teeth "v", "w", "x", "y" and "z" appear as described by WILSON and HILL.

I find evidence of only the one tooth belonging to a milk dentition, namely the nodule "dv"; it is present in both jaws. The significance of the epithelial bodies which WILSON and HILL looked upon as degenerated rudiments of milk teeth I will discuss a little later.

My conclusions are that the complete dental formula of *Ornithorhynchus* should read as follows: $i \frac{0}{5} c \frac{1}{1} pm \frac{2}{2} m \frac{3}{3}$. This is shown diagrammatically in fig. 10.

V—RATE OF GROWTH OF THE DENTAL LAMINA AND OF INDIVIDUAL TEETH

The length of the main portion of the dental lamina of the upper jaw in the younger specimens is given in the following table:

Specimen	Snout-tail length (mm.)	Dental lamina (mm.)
WW	28	0.8 (approx.)
X	56	1.56
Delta	80	2.6
XXVIII B	122	3.09
H.N.	140	4
H.J.	170	5.24

From this it will be seen that there is a steady growth in length of the dental lamina, the rate of growth corresponding very closely to that of the body generally as indicated by the total lengths of the specimens.

In fig. 11 the dental laminae of these specimens are shown; they are all drawn to the same scale so that growth changes can be readily observed. Since the extent to which the incisor region is developed is variable, I have taken "*f*", the canine tooth, as the fixed point in my drawings.

I should like to draw attention to one or two points:

1—Growth is occurring chiefly at the posterior end of the lamina where the molar teeth are being differentiated. In all the specimens the posterior end of the dental lamina is deep and bulky.

2—In addition to this general backgrowth there is a considerable amount of intrinsic growth in the lamina as may be seen from the lengthening of the distance between "*w*" and the upper canine, or between "*w*" and "*y*".

3—There is apparently scarcely any intrinsic growth in the premaxillary or incisor region of the lamina after the tooth germs have been differentiated as in Delta. The distance between "*d*" and "*f*" remains approximately the same in later stages.

4—If the relative position of the upper and lower enamel organs is compared in H.J. and H.X. it will be seen that there must be a considerable differential rate of growth in the two jaws. In H.J. and in the preceding stages (fig. 11), "*w*" lies opposite the middle of "*x̄*", and the enamel organ of the upper "*x*" is almost wholly posterior to that of "*x*" in the lower jaw. In H.X., however, "*w*" lies anterior to "*x̄*", and the two main cusps of the upper "*x*" clearly interlock with the corresponding main cusps of "*x*" in the lower jaw though still lying posterior to them (fig. 81, Plate 44). The enamel organs of the lower teeth therefore grow back relative to those of the upper teeth in the later stages.

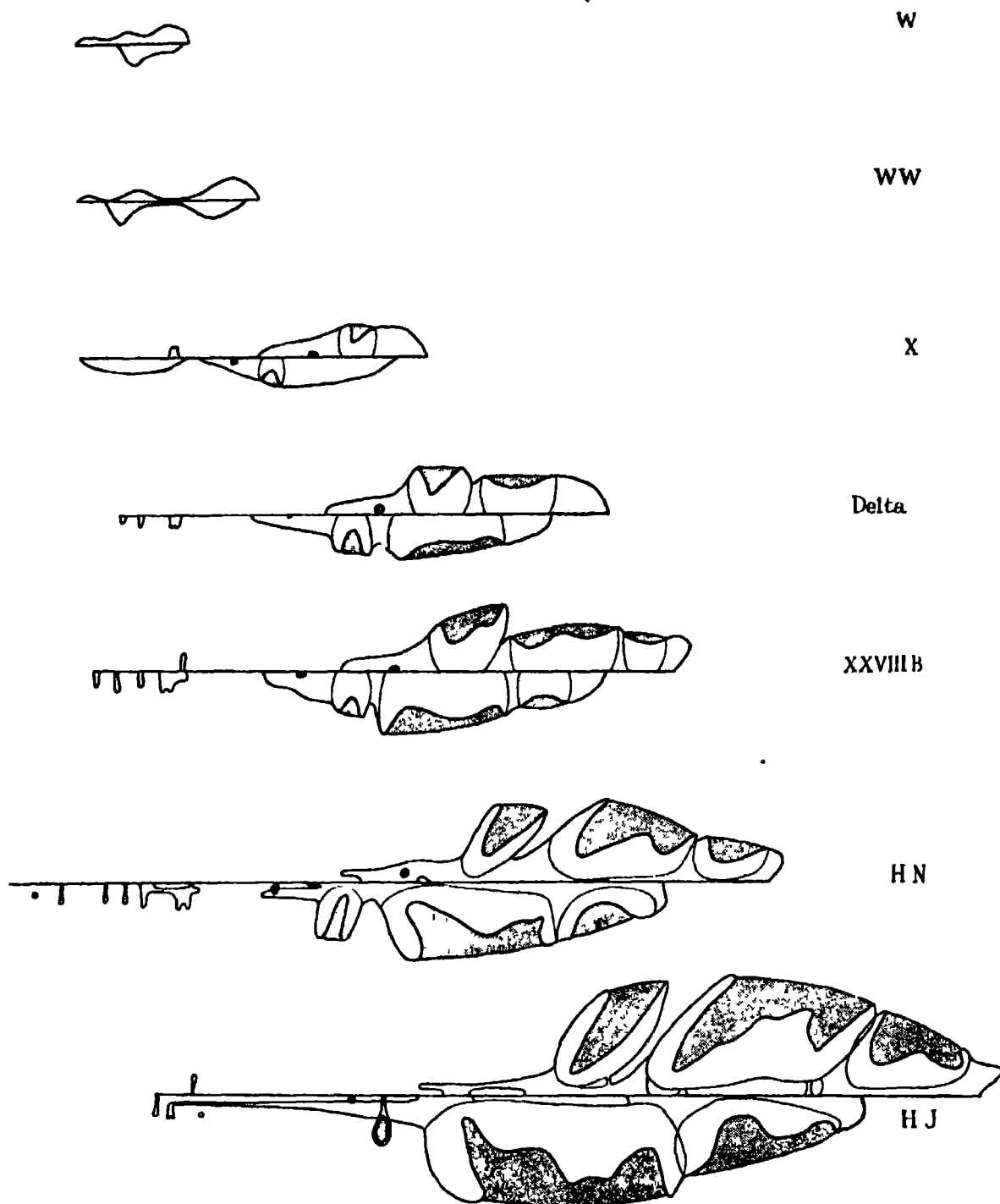


FIG. 11—Graphic reconstructions of the dental laminae and enamel organs of specimens W, WW, X, Delta, XXVIII B, H.N. and H.J., magnified to the same scale so that rates of growth and differentiation of enamel organs can be seen. For the identification of individual enamel organs reference should be made to figs. 1, 2, 3, 5, 6 and 7. $\times 16.5$.

The antero-posterior lengths (in mm.) of the crowns of the two larger teeth in the various specimens are given in the following table:

	Delta	XXVIII B	H.N.	H.J.	H.P.	Beta	H.X.	Simpson			Stewart
								A	B	C	
Upper <i>x</i>	0.68	0.97	1.1	1.8	2.2	3.5	3.7	3.9	4.7	4.1	4.4
Upper <i>y</i>		0.33	0.43	0.88	1.1	2.8	3.3	4.2	4.7	4.6	5.1
Lower <i>x</i>	0.93	1.2	1.2	1.8	2.0	4.0	3.7	3.8	5.1	4.6	5.0
Lower <i>y</i>		0.3	0.5	1.2	1.2	3.0	3.3	3.3	4.8	4.6	4.8

With data from so few specimens available it is clearly unwise to attempt to draw any general conclusions, but it is of interest to notice that the teeth of specimens B and C, and also of Stewart's specimen are of considerably greater length than are the teeth of specimen A, despite the fact that in specimen A the teeth had already erupted.

It had been generally accepted that the diameters of teeth cannot increase after eruption has occurred until DONALDSON and FRENCH (1927) claimed to prove an increase in size of the crowns of the molars of albino rats after they had erupted. BEUST (1930) showed the same thing in the teeth of the pig. It is known that there is some sort of metabolic activity in the dentine of erupted teeth even in man (FISH 1933, p. 49). H. E. and F. D. WOOD (1931) deny that any diametric growth occurs in the molars of the rat after eruption. Too few data are available to settle the question, though it would seem improbable, *a priori*, that any such growth can take place.

VI—THE MORPHOLOGY OF THE TEETH

The morphology of the erupted teeth of *Ornithorhynchus* has been described in some detail by SIMPSON (1929). I will compare the conditions present in my younger specimens with his description. In fig. 12 is reproduced SIMPSON's figure of the teeth in his three specimens.

As the lower incisors and the canines are only represented by vestigial parts of the dental lamina which are soon absorbed, their morphology must remain unknown.

The first upper tooth, "*w*", has been described by SIMPSON as "a single high, slender, sharp cusp". WILSON and HILL refer to its "relative size and simplicity". POULTON (1889) says: "There is one chief cusp, and apparently a second smaller one, externally placed . . . ; but I cannot feel very sure about the latter . . ." One of his figures of a section through this tooth shows the second cusp distinctly. I have already shown that, at the height of its development, "*w*" has three cusps, the main one which is retained being antero-medial in position, and in addition there are smaller anterior and posterior lateral cusps (fig. 9). In H.X., though these latter cusps have been lost, the base of the tooth is still expanded laterally. This temporary appearance of distinct and well-calcified cusps which disappear again so rapidly in ontogeny shows how extremely difficult, if not impossible, it is to reconstruct the phylogenetic history of the monotremes on the basis of cusp development in the erupted teeth.

The second upper tooth, "*x*", is constricted transversely and a deep transverse basin is present. The constriction is particularly marked on the medial border of the tooth

between the two large cusps. From the base of the two main cusps ridges run laterally towards the lateral cingulum; the anterior cusp has a single crest, the posterior has two, thus making it a crescentic cusp. These crests only become prominent as calcification proceeds; they are not apparent, for example, in Beta. The lateral cingulum remains undifferentiated until it commences to calcify (compare Beta and H.X.), and then small cusps appear on the lateral border. SIMPSON says that these are "so variable in number and in prominence as to show no constant plan". I think that, though variable

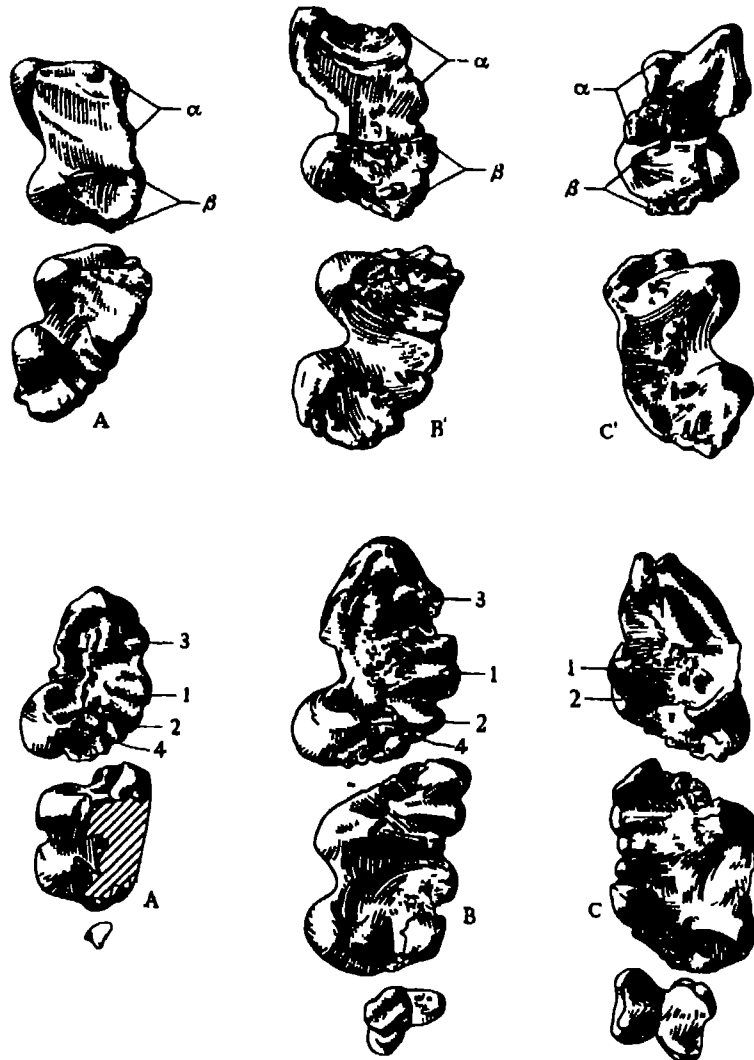


FIG. 12—A reproduction of SIMPSON'S fig. 1 showing the crowns of the teeth of three specimens of *Ornithorhynchus*. A, 8½ weeks, B, 10 weeks and C, 11 weeks old. A', left upper teeth. A, left lower teeth. B', left upper teeth. B, left lower teeth. C', right upper teeth. C, right lower teeth. × 6. I have indicated in this figure the smaller cusps which appear to be fairly constant in tooth "x" of both jaws. Compare with fig. 47, Plate 39; fig. 68, Plate 42; fig. 84, Plate 45; fig. 86, Plate 46.

accessory cuspules are present, four more or less constant cusps can be recognized laterally, there being two opposite each main medial cusp (fig. 86, Plate 46; fig. 12). Thus the basal plan of the tooth would be a double trigone.

In the third upper tooth, "*y*", a similar general plan is followed, but in this case there does not seem to be any constant arrangement of the cingular cusps. The crenulation of the lateral, anterior and posterior margins of the tooth is more marked than in "*x*". The order of development of the parts of the tooth is well shown by comparing the models of H.P., Beta and H.X. In H.P. the antero-medial cusp is already prominent, while the postero-medial one is just developing as a low, rounded swelling (figs. 56, 57, Plate 40); the tooth is narrow transversely, the crown not yet having expanded to form a lateral cingulum. In Beta this expansion has occurred and a raised, though as yet uniform, cingulum is present (fig. 76, Plate 43). In H.X. the cingulum has become cuspidate (fig. 86, Plate 46).

The first lower tooth, "*x̄*", differs from the other large teeth in that it is triangular rather than quadrate in shape. The blunted apex of the triangle is formed by the very large anterior cusp on whose posterior surface there is a swelling which runs back in the middle of the crown to terminate at the median transverse basin (fig. 48, Plate 39; fig. 84, Plate 45). At the postero-lateral angle of the crown is the second main cusp which is already present and starting to calcify in H.P. Subsidiary cusps are carried on the medial cingulum and on the posterior margin of the tooth. I again disagree with SIMPSON when he says that "none are at all constant". Two are, I think, always present, one in the middle of the medial cingulum opposite the basin, and the other just posterior to it opposite the postero-lateral cusp. These two cusps are already seen arising in H.P. (fig. 49, Plate 39) and can be traced through the other specimens (1 and 2 in fig. 68, Plate 42; fig. 84, Plate 45; fig. 12). Two other cusps tend to be present more or less constantly, the one near the base of the main anterior cusp, and the other close to the base of the main posterior cusp (3 and 4 in fig. 68, Plate 42; fig. 84, Plate 45; fig. 12). The presence of three cusps in a transverse row on the posterior part of the tooth (figs. 68, 69, Plate 42; fig. 12) seems to be one of the only points in which anything approaching a multituberculate type of tooth can be recognized.

The second lower tooth, "*ȳ*", again shows two main lateral cusps. The anterior one is prominent, but the posterior tends to lose its apical portion and so become lower and more rounded. Transverse ridges or crests run from these two cusps towards the medial margin. There is a large transverse median basin, but the tooth is not so constricted here as are the upper teeth. One other cusp is apparently quite constant at the antero-medial angle of the tooth; it is present in all the specimens that have so far been figured. The medial cingulum carries four other cusps which also seem to be reasonably constant. As in "*x̄*" there is a tendency for a cusp to develop between the cingulum and the main postero-lateral cusp (fig. 72, Plate 42; fig. 82, Plate 45; fig. 12).

The third lower tooth, "*z̄*", is small and varies in the extent to which it is developed. It tries to repeat the structure of the preceding teeth and develops either (1) a single

antero-lateral cusp (fig. 12 A) or (2) an antero-lateral and a postero-lateral cusp with (a) a medial cingulum developed only anteriorly (fig. 82, Plate 45; fig. 12 B) giving a triangular tooth, or with (b) a medial cingulum developed along the whole length of the crown giving a quadrangular tooth (fig. 12 C).

As SIMPSON states, the teeth are all separate and well spaced from each other. He comments on the observation of WOOD JONES (1923) that in later stages the three teeth of the lower jaw "are fused together into a common calcified mass. This common dental mass, unlike true functional teeth, is extremely brittle in its composition", and suggests that confirmation of this "remarkable condition" is desirable. The figure which WOOD JONES gives of the lower jaw with the teeth *in situ* (1923, fig. 32) shows the first lower tooth, " \bar{x} ", not only as a typical quadritubercular tooth, which is unlike the condition described by any other author, but also as being almost 10 mm. long, which is practically twice the length of the tooth in all the other specimens which have been described and figured.

VII—THE RELATIONSHIPS OF THE MONOTREMES

Directly after POULTON (1888) had first described the presence of true teeth in *Ornithorhynchus*, COPE (1888) suggested that these teeth were multituberculate in type and so tended to relate the Monotremata to the Multituberculata.

This view has been widely adopted. Reproducing THOMAS's figure, TOMES (1923) says on p. 359, "The crown pattern of these teeth are (*sic*) strikingly like those of the fossil *Microlestes* . . ." Later, however, on p. 361, TOMES states that the enamel of a tooth of *Microlestes* does not present the peculiarities of structure found in *Ornithorhynchus*.

OLDFIELD THOMAS in his paper (1889) says on p. 130, "but unfortunately the most careful search among other animals, fossil and recent, mammalian and reptilian, fails to reveal any teeth quite corresponding to those of *Ornithorhynchus*". He says that he is more and more inclined to COPE's suggestion as to the monotrematous nature of the Multituberculata. He gives a figure of a tooth of *Microlestes*, and with reference to it says "it must be insisted that the resemblance between the Multituberculate- and the *Ornithorhynchus*-teeth is of the most general character, and that the two are certainly widely separated genetically, even if we do admit that they appear to possess a relationship nearer to each other than to any other known groups of mammals".

OSBORN (1907), commenting on COPE's multituberculate theory, says on p. 105, "When critically examined, however, the molars of *Ornithorhynchus* are found to be very degenerate both in structure and in pattern, and it cannot truly be said that they actually resemble those of any Multituberculate in the strict sense, because all the higher Multituberculates exhibit an extremely regular mechanical disposition of the cusps, whereas in this living Monotreme the cusps are extremely irregular." The only origin which OSBORN suggests is the very tentative one of derivation from a tritubercular type: p. 107, "It does not appear that the *Ornithorhynchus* molars can be cited as

evidence either for or against the tritubercular theory because of the evidently secondary and largely degenerative changes which they have undergone; they bear evidence of descent from a more primitive regularly cuspidate condition." A little later he goes on: "It is especially noteworthy (1) that unlike the Multituberculates the lower molars reverse the pattern of the upper molars (as in tritubercular teeth generally) and (2) that the highest cusps are on the inner side of the upper molars and on the outer side of the lower molars. So far as these facts are of value they would support the hypothesis that these are degenerate tritubercular teeth." The last sentence is in italics.

AMEGHINO (1908) attempted to relate the monotremes to the edentates.

ABEL (1926) has recently emphasized the relationship of the monotremes to the multituberculates in his description of *Desmostylus*, a Miocene mammal, which he claims is related to both these groups. On p. 136 he says, "Ich habe im Jahre 1922 wahrscheinlich zu machen versucht, dass wir in *Desmostylus* einen Angehörigen des Multituberkulatenstammes zu erblicken haben. Mit dieser Auffassung steht allerdings in Zusammenhang, dass ich die Multituberkulaten als mit den Monotremen in engstem genetischen Zusammenhang stehend betrachte, so dass Multituberkulata und Monotremata einen geschlossenen Stamm bilden, von dem das Schnabeltier und die Schnabeligel bis in die Gegenwart hereinreichen." He continues later: "Die Monotremen sind vielleicht in früherer Zeit in viele Stämme gespalten gewesen, von denen wir bisher nur die letzten Ausläufer in der Gegenwart und die wenigen fossilen Multituberkulaten kennen; vielleicht ist das eigentliche Entwicklungszentrum der Monotremen gerade in der pazifischen Region zu suchen. . . . *Desmostylus*. . . Er weist die meisten Beziehungen zu den fossilen Multituberkulaten und zu den lebenden Monotremen auf, hat aber auch einzelne Merkmale, die auf eine Verwandtschaft mit diprotodonten Beutlern hinweisen." Other authors do not agree that *Desmostylus* is related either to the multituberculates or to the monotremes.

SIMPSON (1929) considers the possibility of relationship of the monotremes to the various known groups of Mesozoic mammals and shows that any such relationship is improbable. He concludes that "a vague resemblance to the triconodonts may eventually prove to be significant but at present is not trustworthy". The evidence he says "tends to emphasize the rather widespread opinion that *Ornithorhynchus* is not merely a more primitive therian or even one specialized on the general primitive therian basis, but something quite distinct". He is driven to suggesting a hypothetical origin independently from a mammal-like reptile, which could give rise to such teeth as those of *Ornithorhynchus* "by the action of processes which did occur in the theromorphs".

Recently, GREGORY (1934, p. 262) states that SIMPSON's figures (1929) suggest to him a possible derivation of the teeth of *Ornithorhynchus* by excessive degeneration from a "somewhat *Caenolestes*-like stage". A little later on I show that I agree with SIMPSON in considering any derivation from a tritubercular type of tooth to be most unlikely. GREGORY goes a step farther and puts forward the "tentative hypothesis" that

Ornithorhynchus is an extremely specialized derivative from the Australian phalangeroid stem. He suggests that the "beak" of *Ornithorhynchus* represents an enlargement of such a rhinarium as is seen in *Phascolarctus*, and he compares the double "V" pattern of the upper molars with that of *Phascalomys*. He dismisses the "reptilian" characters of, for example, the reproductive organs of *Ornithorhynchus* by saying that they have arisen "from a neotenuous arrest of ontogenetic phases that are transient in the diprotodonts". I fail to see any material evidence in favour of such a view; it can surely only be looked upon as a despairing attempt to draw *Ornithorhynchus* into the "recognized" mammalian fold.

I have purposely given a *résumé* of the views put forward by various workers because one is struck by the way in which, no matter how great the attempt to "fit in" the teeth of *Ornithorhynchus*, not a single author has been able to satisfy even himself that there is any convincing evidence for close relationship with any other mammalian form.

This is not really so surprising as might be imagined because the extent of the degeneracy of the teeth can only be fully appreciated by studying the histology of the later developmental stages. The main points in connexion with this have already been mentioned when describing specimen H.X. What with the retrogressive changes in the main cusps, the ameloblastic strands extending from apparently subsidiary cusps which may therefore well have had a much greater importance in earlier phylogenetic stages, and the extremely degenerate state of the roots of the teeth (to be described later), it is clearly unreasonable to expect to trace any true relationships.

TOMES (1923) says that as one approaches the roots of the teeth an abrupt transition in dentinal structure takes place, all the dentinal tubes disappearing and large lacunae appearing. "Thus the dentine structure of the tooth is somewhat that which we are accustomed to see as a result of pathological processes, and would suggest, as far as it goes, that the *Ornithorhynchus* tooth has degenerated from some earlier and more complete tooth-form in which the roots consisted of properly developed dentine."

Similarly, WOOD JONES (1923) says, "Even if we admit a similarity between the form of the molar teeth of the Multituberculata and those of *Ornithorhynchus*, we must remember that, in the case of the living animal, we are dealing with teeth which are in a very degenerate condition, and are, therefore, not necessarily typical of the dentition of the ancestral Ornithodelphian."

It is impossible to surmise what the crown pattern of these teeth might have been before degeneracy set in, with the result that phylogenetic speculations would appear to be useless. The vast palaeontological gap cannot be bridged with any feeling of security until more early mammalian fossil material is available. It is generally agreed that the Monotremata represent an extremely ancient mammalian stock; GREGORY in his "Orders of Mammals" (1910) says, "The ancestral lines of the Marsupials and Monotremes converge into a common source which had already acquired many essential mammalian characters." A solution to the origin of monotreme teeth can only be

found by extending considerably the present range of knowledge of variations in tooth form to be met with in the mammal-like reptiles and in the Mesozoic mammals.

Nevertheless, taking the teeth of *Ornithorhynchus* at their face value, it is interesting to see how far their pattern can be said to resemble that of other mammalian teeth. For a more detailed review of the possible derivation of monotreme teeth, SIMPSON's paper (1929) should be consulted.

The Mesozoic mammals are classified according to the types of teeth they possess. There is the group which possesses teeth of the tuberculosectorial type; this group includes the Pantotheria of the Jurassic and their probable descendants, the marsupial and placental mammals found in the Upper Cretaceous. The other groups are (1) Symmetrodonta, (2) Triconodonta and (3) Multituberculata (including the Microcleptidae).

If the monotremes are to be related at all closely to the marsupials and to the placentals, it would be expected that their teeth would be derivable from a tuberculo-

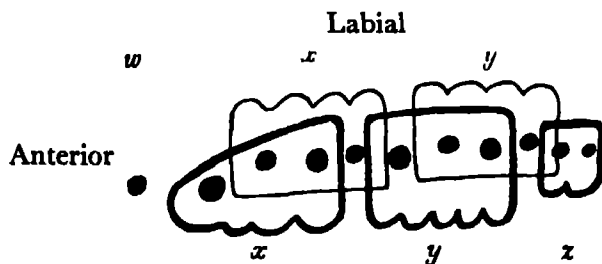


FIG. 13.—Diagrammatic drawing to show how the teeth of *Ornithorhynchus* are related to each other in occlusion. The teeth of the lower jaw are heavily outlined and their main cusps indicated by black dots. The main cusps of the upper teeth are hatched.

sectorial type. This was suggested by OSBORN as a possibility. Such a possibility is tenable only with great difficulty, as so many modifications of the fundamental tri-tubercular pattern are required.

From the type of occlusion which is present in *Ornithorhynchus* and which is diagrammatically represented in fig. 13, the main antero-medial cusp of the upper teeth must be the protocone, as SIMPSON suggests, since it bites into the basin of the lower tooth which must correspond to the talonid of a tuberculosectorial lower molar. The postero-medial cusp would then be the hypocone, and the paracone and metacone would only be represented by the several cusps on the lateral cingulum. In Beta there is a suggestion of two elevations on the lateral border of the tooth which might possibly correspond to the paracone and metacone, in which case in later development each of these would have split into two to give the mature arrangement of four cusps on the lateral cingulum. This is very unlikely, especially as the paracone is the first cusp to develop ontogenetically in the upper molars (TAEKER 1892; RÖSE 1892; ADLOFF 1903 *b*), whereas in *Ornithorhynchus* the first cusp to arise is the antero-medial one, the cusps on the lateral border appearing much later.

In the lower teeth a possible theoretical resemblance to the typical tuberculosectorial arrangement can be made out if it can be supposed (*a*) that the smaller cusps are in fact more or less constant, and (*b*) that these cusps originally possessed a greater significance than they do now. The main cusps would be the protoconid and the hypoconid.

However, these homologies are extremely unlikely; the early ontogenetic appearance of the hypocone (and indeed its appearance at all in such a primitive form), and the reduction to such a degree of the paracone and metacone, make a derivation from the tuberculosectorial type very improbable.

The symmetrodont teeth bear no relation to those of the monotremes. The Symmetrodonta have a single, tall, medial cusp in the upper molars, with accessory anterior and posterior cusps carried on its lateral slopes, and vice versa in the case of the lower teeth. They are interlocking and shearing teeth, whereas the teeth of *Ornithorhynchus* are essentially for crushing.

Triconodonta possess three cusps arranged longitudinally and a narrow cingulum, medial in the case of the lower teeth, which may be crenulated. They are purely shearing teeth, and again there is no evidence for relationship with the teeth of the monotremes.

The typical Multituberculata have teeth which are utterly unlike those of *Ornithorhynchus*; they are characterized by having two or three rows of cusps separated by straight longitudinal grooves, quite unlike the two main cusps and the transverse groove of the monotreme.

The Microcleptidae, of the late Triassic, have generally been grouped with the Multituberculata, but, according to SIMPSON (1928), "the affinities of the Microcleptidae are painfully uncertain". Certainly the teeth are unlike those of other Mesozoic mammals. With their few and prominent cusps on one side and a cuspidate arrangement on the other, they agree in a general way with the teeth of *Ornithorhynchus*, as may be realized by referring to SIMPSON'S (1928) figs. 15 and 17 of *Microcleptes fissurae* and *Thomasia anglica*. The longitudinal valley of the microcleptid molar forms the most noticeable difference. The tooth of *Microlestes*, figured by THOMAS as the nearest approach he could find to the monotreme condition, belongs to this group.

On the whole my conclusion is that, though a tuberculosectorial derivation might, on general grounds, be reasonably expected in the monotremes, and with some stretch of imagination is a conceivable possibility, the nearest relationship from the point of view of tooth morphology would appear to be with the microcleptid group of mammals. It must be admitted that there is nothing convincing in any of these relationships. SIMPSON (1928) says on p. 183, "There is nothing really remarkable in this lack of ancestral monotremes. Throughout the Tertiary, and perhaps for some time before that, the group has probably been Australian, and the pre-Pleistocene mammalian life of the Australian region is still virtually unknown. The known Mesozoic mammals are extremely few in number, and the chances in any event would be strongly against

any of them being related to a small and isolated group like the Monotremata. Even in the Mesozoic Era Asia would be the most probable place to look for ancient monotremes. Only five species of Mesozoic mammals are known from Asia, and these are from well up in the Cretaceous."

VIII—THE ROOTS OF THE TEETH

Very little mention has been made of the roots of the monotreme teeth. TOMES (1923) says that there are short stunted roots which hold the teeth for a time fairly firmly in position. He also mentions that the curiously cupped and sculptured surface of the horny plates has its form determined by once having formed the bed for a tooth with several roots. TOMES further states that the roots are of softer, coarser material than the crown, and that this degeneration of the dentine near the root portion of the tooth is not approached in any other mammalian tooth. "In those teeth which have no roots, if such an expression may be allowed, but which are about to become ankylosed to the bone, something of the kind may be seen."

POULTON (1889) says on p. 26, "Furthermore, Professor SEELEY's suggestion that 'there is a certain relation . . . between the complexity of the crown and the complexity of the fangs' is extremely probable, and leads us to conclude that the developed teeth of *Ornithorhynchus* must have possessed many fangs."

STEWART (1892) gives drawings of the deep surfaces of the teeth which show a complex arrangement of roots.

The question has recently been raised by ORBAN and MUELLER (1929) as to the method of formation of the several roots of a multi-rooted tooth. It used to be thought that, after the crown of the tooth had been formed, Hertwig's epithelial sheath grew in horizontally at certain points to effect the necessary subdivision of the single opening of the pulp cavity. They showed clearly by examining the teeth of developing rats from 5 days before birth up to 20 days old, that the plan of the division of the roots is determined long before the completion of the crown of the tooth, in fact it is recognizable before any dentine has been formed on the crown. The outlines of the roots are foreshadowed first by an eccentric expansion of the basal opening of the enamel organ. During this expansion some parts of the edge of the epithelial sheath of Hertwig remain relatively fixed, and these parts thicken and grow together to determine the number and the arrangement of the roots. ORBAN and MUELLER made graphic reconstructions of the base of the enamel organs to show the outline of Hertwig's sheath and the outline of the opening bounded by it; they called the latter the "basale Öffnung des Keimes".

Using the same method I have made graphic reconstructions of the basal openings of the enamel organs of teeth "x" and "y" of the lower jaw of *Ornithorhynchus* at two stages of development, namely, in specimens H.P. and H.X.

The enormous complication of the root plan in the later stages of development is obvious from these diagrams (fig. 14). In fact, I have had to leave out several of the

more minute openings, since in one or two places they become so numerous and lie so close together as to give the appearance of a finely fenestrated membrane to the epithelial sheath. In both teeth there is apparently a tendency to retain a single large opening into the pulp cavity at the posterior end of the teeth, this opening extending over most of the transverse diameter of the base of the tooth. Over the rest of the tooth, however, the growth and fusion of various parts of the epithelial sheath results in the basal opening of the enamel organ (which is still relatively simple in tooth "y" of H.P.) being cut up into a large number of smaller openings. This affords a further example of the degeneracy of the teeth of *Ornithorhynchus*.

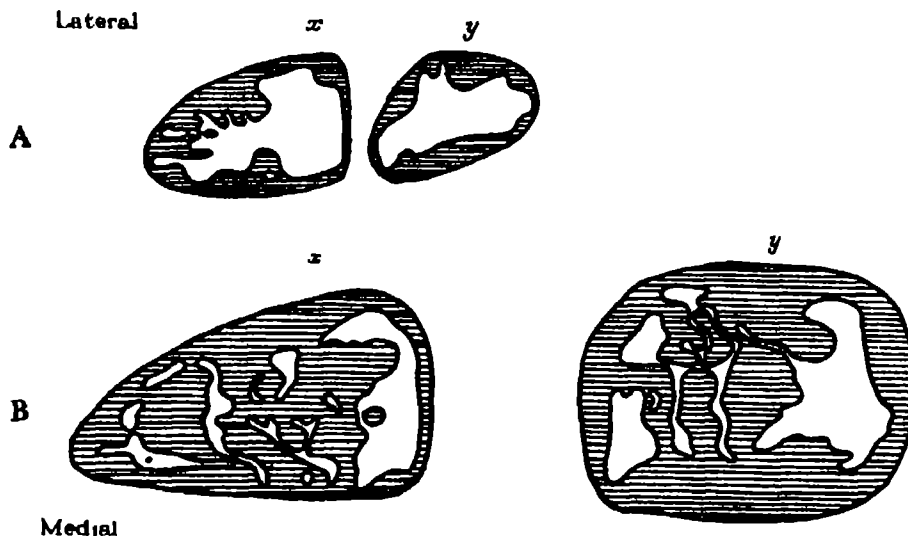


FIG. 14—Graphic reconstruction of the basal opening of the enamel organs of the lower teeth "x" and "y". A, specimen H.P.; B, specimen H.X. The hatched portions represent Hertwig's epithelial sheath, and the spaces are the gaps left whereby communication is retained between the pulp of the tooth and the surrounding mesoderm. $\times 16.65$.

IX—VASCULARITY OF THE ENAMEL ORGAN AND THE FUNCTIONS OF THE STELLATE RETICULUM

In his "Manual of Dental Anatomy" (1923) TOMES says on p. 157, "So simple a matter as the vascularity or non-vascularity of the enamel-organ is not yet settled." With reference to this it may be said that, though a number of authors have denied the presence of blood vessels in the enamel organs of the forms they have examined, later workers have almost unanimously agreed that the stellate reticulum does become vascularised at an earlier or later stage of tooth development in the mammals.

The following are among those who have denied the existence of blood vessels in the enamel organ. WEDL (1870) said that the enamel organs of the human incisors were avascular. LEGROS and MAGROT (1879), speaking of the mammalian enamel organ, said (p. 271), "Sa coloration est gris clair, ce qu'il doit en partie à l'absence complète de vaisseaux sanguins, cet organe étant, comme nous le verrons plus loin,

absolument dépourvu aussi bien de système vasculaire que de système nerveux." KLEIN and NOBLE SMITH (1880) found no blood vessels in the stellate reticulum of the dog; similarly SUDDUTH (1886) could find none in injected specimens of the pig. PAUL (1896) uniformly failed to find any vessels in the enamel organ of the calf or the lamb. TOMES said (p. 158) that in the very large numbers of enamel organs he had had under observation, he had never seen a blood vessel which appeared to him to be unquestionably in the enamel organ. SKILLEN (1921) found no vascularity of the enamel organ in the pig, cat, or dog. Most recently, JORDAN (1921) could not find any vessels entering the enamel organs of rodent incisors, and in newborn kittens the vessels only invaginated the external enamel epithelium to a slight degree and did not actually perforate it. Later (1923), the same author found blood vessels to be constantly present in the enamel organs of the molar teeth of rats. The only exceptions he admits to the general rule of avascularity of the mammalian enamel organ are afforded by monotremes, marsupials and the molar teeth of rats and mice. When vascularity is present he suggests that it is of no advantage to amelogenesis but rather otherwise, as the red blood corpuscles so introduced into the stellate reticulum appear to cause a reaction of the latter as though the corpuscles were foreign substances.

On the other hand, many observers have shown that the mammalian enamel organ does become vascularized. POULTON (1889) remarks of *Ornithorhynchus* on p. 17, "it is quite certain that blood vessels are present in this layer (i.e. the stellate reticulum) and that they extend into all parts of it": he mentions also that he finds abundant vessels in the rat and says, "It is very extraordinary that the existence of such obvious vascular channels should have been denied." HOPEWELL-SMITH and MARETT TIMS (1911) refer to the undoubted vascularity of the enamel organ of *Macropus billardieri*; they could only trace vessels as far as a point midway between the external and internal enamel epithelia. These authors suggest that this unusual vascularity is correlated with the precocious development of enamel which in *Macropus* is either deposited simultaneously with, or even precedes, the calcification of the dentine. BOLK (1915) described vascularization in *Phascolarctus* and later (1929) to an even more marked extent in *Trichosurus*. Again, THORNTON CARTER (1918) showed that capillaries reach as far as the stratum intermedium in *Macropus ruficollis*. Thus the vascularity of the marsupial enamel organ has been well established. In placental mammals the case is not so clear. ADDISON and APPLETON (1922) conclusively showed that in the molars of the rat, multiple blood vessels penetrate into the enamel organ as far as the stratum intermedium but fail to enter the latter. They suggested that these vessels are related to the beginning of amelogenesis. KINGERY (1924) claimed to find vascularization in the teeth of the rat, pig and man; in the two former the vessels penetrated the enamel organ relatively early, whereas the opposite was the case in man. MÜLLER (1927) demonstrated vascular enamel organs in *Dactilomys*. SANTONÉ (1935) related the commencement of enamel formation on the molars of *Cavia cobaya* to the time at which capillaries reached the stratum intermedium. A paper by GULAT (1936) on the

development of rodent incisors has just appeared in which the significant part played by the capillaries in enamel formation is emphasized.

The functions of the enamel pulp (stellate reticulum) have been widely discussed. REICHENBACH (1928) summarizes the generally accepted views of the role of the enamel pulp as (1) to supply nutriment for the formation of enamel, or (2) the purely mechanical function of allowing space for the development of the enamel. He himself denies both these views and considers that the pulp acts as an elastic cushion and so is simply a tissue support. BOLK (1929) also refuses to admit a nutritive function to the enamel pulp, as he shows in *Trichosurus* that over the prominent cusps of the teeth there is no pulp left at a stage before any enamel has started to form. He considers that the invasion of the pulp by blood vessels is for the purpose of destroying the stellate reticulum so that the vessels can get into relation with the ameloblasts as soon as possible. THORNTON CARTER (1918) says that the distinctive appearance of the stellate reticulum is due to the accumulation of metaplastic material in the cells which is used up and elaborated by the receding ameloblasts. ADDISON and APPLETON (1922) point out that the stellate reticulum is only developed over those parts of a tooth on which enamel is deposited, but that on the other hand the reticulum is not essential for enamel formation since it is not present in the mature rodent incisors, nor in the teeth of fish, Amphibia and most Reptilia. They think that the enamel pulp is not primarily concerned with amelogenesis but gives the space which is required for the expansion of the crown of the tooth. KINGERY (1924) suggests that the stellate reticulum has an important nutritive function in those animals in which vascularization of the enamel organ occurs at a late stage; he thinks that in such cases (e.g. man) a papillary layer is formed by modification of the external enamel epithelium to increase the surface for absorption of amelogenetic substances from the vessels which surround the enamel organ.

GULAT (1936) thinks that any attempt to bring the enamel pulp into physiological relationship with the secretory activity of the internal enamel epithelium is invalidated by the rodents, in which enamel is added for years after the enamel pulp has disappeared. He says that the position of the future germ of an incisor tooth can be recognized in the rodent by the localized vascularity of the mesenchyme which precedes the formation of the enamel organ. The capillaries in the rodents penetrate, not merely as far as, but actually into the stratum intermedium and so get into the closest possible contact with the ameloblasts. GULAT favours a mechanical function for the stellate reticulum. On p. 388 he says, "Der Name 'Schmelzorgan' besteht ja nicht ganz zu Recht, da er zu eng gefasst ist. Es dient auch hier den Hartschubstanzen als Widerlager, als plastische Gussform; denn gerade die Zahnform ist eine der wichtigsten Bedingungen für den ganzen Kauakt": and on p. 392, "Der Zeitpunkt, in dem die Kapillaren das Stratum intermedium erreicht haben, scheint einer der wichtigsten der ganzen Zahnentwicklung zu sein. Nirgends sah ich eine Schmelzablagerung, bevor dieses Stadium erreicht war. Das Vorhandensein der Kapillaren scheint also eine physiologische und morphologische Vorbedingung für die ganze Adamantogenese zu sein."

There can be no question about the vascularity of the enamel organ of *Ornithorhynchus*; blood vessels invade the stellate reticulum first of all in the neighbourhood of the main cusps, that is, those parts of the crowns of the teeth where enamel formation commences. These vessels penetrate the external enamel epithelium at several points and grow inwards until they meet the stratum intermedium. The general vascularity of the enamel organs at a late stage of development can be well seen in fig. 81, Plate 44. Fig. 45, Plate 38 shows a single blood vessel entering an enamel organ at an earlier stage.

It would seem that in the case of *Ornithorhynchus* enamel formation is dependent on the close relationship of the capillaries with the stratum intermedium, as GULAT found in rodents. In figs. 98, 99, Plate 49, photographs of sections of the same tooth are shown; around the main antero-medial cusp a thick layer of enamel is deposited, and the very numerous blood vessels of the enamel organ are seen to reach the stratum intermedium; on the other hand, in that part of the crown where no calcification has yet occurred (fig. 99, Plate 49), the blood vessels, again numerous, are seen to be separated from the stratum intermedium by a thick layer of as yet unvascularized stellate reticulum.

So far as can be judged, it would seem that the stellate reticulum has no more than a mechanical function in the monotremes; the secretory activity of the ameloblasts would appear to be dependent upon the nourishment brought to them by the invading capillaries.

X—THE EPITHELIAL NODULES AND THEIR SIGNIFICANCE

POULTON (1889) mentioned the presence of epithelial nodules in the enamel organ of *Ornithorhynchus* and said that further investigation upon them was required. He described these nodules in the following way (p. 19): "One peculiarity of this layer (stellate reticulum) is the presence of an epithelial nodule situated just beneath the outer layer of the enamel organ, almost immediately over the apex of each calcified cusp of the second and third tooth (i.e. 'x' and 'y'). Nothing of the kind could be made out in the case of the first upper tooth (i.e. 'w')... In some cases there was the appearance of an epithelial cylinder extending from the nodule towards and perhaps reaching the stratum intermedium or enamel cells over the apex of the cusp. It seems clear that the nodule is in some way associated with the chief cusp, for there was always a nodule above each of the latter, while they were never found elsewhere... the inner cells appear to be corneous and collected into a dense central mass, between which and the outer fusiform cells is a space containing loosely-packed cells resembling the former in character." He further defined their position as being at the extreme edge of the stellate reticulum, and his figures show them as lying inside the external enamel epithelium.

MARETT TIMS (1899) described a structurally similar type of nodule in relation to some of the cheek teeth of *Cavia*; he referred to these nodules as "concentric bodies"

and suggested that they were the last vestiges of milk teeth. The same author had previously described such a body in relation to the last upper premolar of the dog (1896), and mentioned that WOODWARD had found a similar body in the same situation in *Gymnura*. Later (1903), in discussing the evolution of the teeth in the mammals, MARETT TIMS said, "In the concentric epithelial bodies of *Cavia*, *Canis*, *Gymnura* and *Ornithorhynchus* we have, I believe, the last traces of a vanishing dentition which must have preceded the cheek-teeth on account of their labial position." He had thus assumed that the nodules described by POULTON were of the same significance as those in the guinea-pig and dog.

WILSON and HILL (1897) in describing the development of teeth in *Perameles* mentioned that epithelial "nests" or "pearls" were formed close to the tooth cusps in a late stage just before eruption; they thought that these cell nests were probably similar to the nodules described by POULTON. When, however, they later examined the nodules in *Ornithorhynchus* WILSON and HILL (1907) concluded that these structures were of an entirely different nature from those they had previously described in *Perameles*; the latter were purely epithelial degeneration products comparable with the pearls found in the median raphe of the palate, whereas the nodules in *Ornithorhynchus* were to be regarded as a series of vestigial representatives of an earlier tooth generation.

HOPEWELL-SMITH and MARETT TIMS (1911) figured a "concentric epithelial body" in relation to the fourth upper premolar in *Macropus billardieri*. This body lies attached to a labial outgrowth of the dental lamina some distance from the neighbouring enamel organ; the nodule which MARETT TIMS described in the dog lies in the same relative position. In neither case is the position of these bodies similar to that which they occupy in *Ornithorhynchus* where they lie inside the enamel organ. The only concentric body described which seems to be comparable in position with those of the monotremes is the one in relation to the most anterior cheek tooth in *Cavia* where it lies "directly in the line of the dental lamina running between the oral epithelium and the tooth".

It is obviously of very considerable importance to determine whether these nodules in *Ornithorhynchus* represent milk teeth or not. If WILSON and HILL are right in their interpretation it would be strong evidence that each functional molar tooth is the equivalent of two or three simple predecessors, in other words, that some form of concrescence had occurred to produce the complicated teeth of the young adult.

RÖSE and KÜKENTHAL strongly promulgated the concrescence theory, and it has been adopted in a modified form by MARETT TIMS and later by BOLK, though the latter preferred to draw a distinction between concrescence and what he termed "concentration". However, the embryological evidence for concrescence is slight, and palaeontological evidence definitely opposes it.

SIMPSON (1929), discussing WILSON and HILL's findings, says that the evidence for concrescence in *Ornithorhynchus* is insufficient because (1) the true nature of the nodules is open to doubt, (2) although related to the cusps in position they do not correspond to them in number, and (3) even if they should be representatives of milk teeth, yet

two or more nodules might represent separated vestiges of a single but complex predecessor. His conclusion is that "The origin of the cheek teeth of *Ornithorhynchus* by concrescence is highly improbable."

BROOM (1935) expresses an opinion about the significance of these nodules. Having said that he does not agree with WILSON and HILL's view, he goes on to say on p. 326: "The enamel organ of a tooth may be regarded as morphologically part of that tooth and of no other tooth, and every structure in that organ seems to me to be part of that one tooth. It is admitted that the teeth in *Ornithorhynchus* are degenerate. Possibly they may be derived from teeth like those of *Tritylodon* with a dozen or more cusps, and these epithelial nodules and the little toothlet in 'y' may be the detached remnants of lost cusps. Remains of an earlier set ought not to be found in the enamel organ, but outside the enamel organ on the labial side of its neck."

The evidence which I have to offer with regard to these epithelial nodules may be considered under four headings.

1—Number and Position

POULTON described the nodules as being constantly associated with the main cusps of the teeth and never to be found elsewhere. WILSON and HILL agreed with this but they found two nodules present in relation to the anterior cusp of "y" in both upper and lower jaws, and therefore, as none was found in relation to "w", five nodules in each jaw were described.

I find that these epithelial bodies may be divided into two groups:

Group A—These nodules are constant in number and position. They form a perfectly regular series, one nodule being present over the main cusps of every cheek tooth. There are five of these constant epithelial bodies on each side of each jaw, though they do not correspond with the five described by WILSON and HILL. They are

$$\frac{dw, dx_1, dx_2, dy_1, dy_2}{dx_1, dx_2, dy_1, dy_2, dz}$$

Actually the lower "w" probably has such a body (specimen H.N.), but the tooth is so degenerate that its nodule would scarcely be expected to appear.

All these nodules are not only in immediate relation to the apices of the cusps but they are also all contained within the enamel organ. WILSON and HILL said that though the nodules may appear to be included in the enamel organ they are really morphologically outside them. I disagree with this. They said that in several cases they could discover an opening or depression in the surface of the enamel organ near the nodule, indicating that it had originally been engulfed from outside. There is certainly no difficulty about finding such openings since the surface of the enamel organ in these later stages becomes fragmentary and the external enamel epithelium is broken up, largely by the entering blood vessels. Nevertheless, I hope to show that in their earlier stages the nodules are not near the surface of the enamel organ, ready to be

engulfed by it, but on the contrary lie much more deeply in the stellate reticulum than they do in later stages. The examination of figs. 37, 38, Plates 36, 37; fig. 63, Plate 41; figs. 93, 94, Plates 47, 48 will show that the external enamel epithelium is continuous over the nodules.

Group B—This is the group of nodules which I have described as being adventitious. These bodies differ fundamentally from those of the previous group in that they are invariably outside the enamel organ. They are sporadic in appearance and are in no way related to the cusps of the teeth. Such nodules are seen in specimens H.P. and Beta.

In H.P. one of these nodules is found in relation to the upper tooth "x"; I have termed it " dx_3 " (figs. 55, 64, Plates 40, 41). It is outside the enamel organ near the mouth epithelium and, if it can be said to be related to the tooth at all, lies over the posterior part of the lateral cingulum. It is clearly not in any sense in series with the other epithelial bodies.

In Beta there are two of these adventitious nodules, and it is unfortunate that WILSON and HILL should have had only this one specimen available for examination. The nodule which they described as " dy_1 " in the lower jaw lies close to the deep aspect of the mouth epithelium and is separated from the surface of the enamel organ by a considerable distance; it is only present on one side of the jaw and is without doubt a detached portion of the mouth epithelium exactly comparable with the body " dx_3 " in H.P. The second adventitious nodule in Beta is the undoubted vestigial toothlet which WILSON and HILL called " dy_2 ". I have already fully discussed this body and will only recapitulate the important points which invalidate its claim to be in series with the true epithelial bodies described in group A:

1—It is outside the enamel organ, recessed into its surface.

2—On the other side of the jaw it is represented, not by a "typical concentric epithelial nodule" (WILSON and HILL), but by an undifferentiated epithelial mass split off from the deep aspect of the epithelium of the mouth and quite separate from the enamel organ.

3—It is unrepresented in any other specimen with the very doubtful exception of H.P., where there is an ingrowth of the mouth epithelium in a similar situation (fig. 80, Plate 44).

4—None of the other epithelial bodies differentiate recognizable tooth structures at any stage.

This group of nodules is then comprised of a total of three examples in all the specimens examined. In each case their position, both in relation to the enamel organ and to the cusps of the teeth, differs from that of the other nodules which are always present in certain constant positions. Also they occur only in isolated specimens, and then usually only on one side of the jaw. Whatever significance they may have, if indeed there is any phylogenetic significance to be attached to them, it is certain that

they are of a totally different nature from that of the epithelial bodies which lie inside the enamel organs in close relation to the cusps.

Another very significant fact is that, in the latest stage examined (H.X.), typical small epithelial nodules are found developing in relation to the apices of the anterior and posterior cusps of the medial cingulum of the lower tooth "y" (fig. 95, Plate 48). These lie just over the cusps and are connected to them by a strand of cells in exactly the same way as the epithelial bodies appear in their early stages over the main cusps of the teeth. Since these bodies are similar in all respects (except their degree of maturity) to the series in group A, and since they lie on the medial side of the enamel organ, it makes it impossible to believe, even apart from other evidence, that any of the nodules belonging to group A can represent vestigial milk teeth.

2—*Developmental Origin*

The epithelial nodules which lie inside the enamel organ and which at later stages are at the extreme edge of the stellate reticulum close to the external enamel epithelium, arise at an earlier stage deeply in the stellate reticulum in close relation to the degenerated apex of a cusp and can be followed as development proceeds to their more peripheral position.

If we take, for example, the nodule " dx_2 " in the lower jaw and trace its development, it will be found to appear first of all in specimen H.J. as a small condensation of darkly staining cells of the stellate reticulum very close to the apex of the postero-lateral cusp of tooth "x" and connected with the cusp by a dense strand of cells. At this stage it is nearer to the cusp than it is to the external enamel epithelium. In specimen H.P., keratinization is commencing in the centre of the darkly staining mass, and it is now found to be in a position approximately half-way between the cusp and the external enamel epithelium (fig. 61, Plate 40). In specimen H.Q. the same body has become completely cornified so that there is no longer a capsule of reticular cells around it, and it is now nearer to the external enamel epithelium than to the cusp (fig. 66, Plate 42); an indication is still present in this specimen of the original connexion of the nodule with the cusp. In specimen Beta, the fully keratinized body has reached the surface of the enamel organ and is immediately under the external enamel epithelium. Finally, in specimen H.X., where amelogenesis has commenced, small islets of enamel may be found in the stellate reticulum along the line of the original connexion of " dx_2 " with the cusp; these may just be recognized in fig. 81, Plate 44.

This is the typical developmental history of all these nodules, and it cannot be doubted that they are derived from the degenerated apices of the teeth. They certainly do not arise outside the enamel organ and become secondarily incorporated in it.

Sometimes there is a definite epithelial (ameloblastic) strand to be found in later stages running from the cusp to the neighbourhood of the epithelial nodules. WILSON and HILL recognized this and remarked on p. 151, "In certain cases it is true that the epithelial strand so constituted does appear to reach and come in contact with the

outer shell of the concentric nodule, but this relationship is not an invariable one, and in all probability is of no essential significance." I believe, on the contrary, that this relationship is fundamental. With the extent of degeneracy which is apparent it is not surprising to find that this epithelial strand is not invariably present, and does not always, when present, actually join the nodule.

3—*Time of Appearance*

If these nodules represent milk teeth one would expect to find some indications of their enamel organs in younger stages, but such is by no means the case.

The milk tooth "*dv*" is already present in the youngest specimen in which any differentiation of the dental lamina has occurred (specimen X), and it would be reasonable to suppose that any other milk teeth in series with "*dv*" would also show some sign of their presence at early stages, certainly as soon as the permanent enamel organs have been formed. Yet, far from this being so, these epithelial bodies do not appear until late stages of development: in specimen H.N. only one nodule is present in each jaw, and the nodules in fact appear only when the corresponding cusps of the permanent teeth are well formed.

Finally, if these bodies represent a series of vestigial milk predecessors, they would almost certainly develop in order from before backwards. But it is found that, with the more rapid growth of the anterior cusps of the teeth, the nodules "*dx*₁" and "*dy*₁" are present at a stage when "*dx*₂" is only just commencing to differentiate (specimen H.J.). Instead of the expected order (if these were originally separate milk teeth) of "*dx*₁", "*dx*₂", "*dy*₁", the order of appearance is actually "*dx*₁", "*dy*₁", "*dx*₂", corresponding to the order of cusp differentiation.

4—*Structure*

The cells forming the epithelial body first of all become corneous and develop a mass of keratin granules. At a later stage the cells in the centre break down and those at the periphery become flattened and concentrically arranged. Eventually a very thin capsule is left with a large centre which is composed of degenerated cells most of which are amorphous.

This sequence can be clearly followed in the case of "*dx*₁" of the lower jaw by referring to fig. 37, Plate 36; fig. 65, Plate 41; fig. 93, Plate 47 in specimens H.N., H.Q. and H.X. respectively.

Taking all these facts into consideration I think that it must be agreed that the epithelial bodies in *Ornithorhynchus* are detached and degenerated portions of what were at one time more prominent cusps. In this respect it is interesting to notice how the main cusps of the teeth of *Ornithorhynchus* remain embedded in the enamel organ and well separated at their apices from the external enamel epithelium as compared with the condition found, for example, in the marsupials where the cusps bulge out the surface of the enamel organ and delete or dislodge the stellate reticulum which originally lay over them (see figs. in BOLK's paper (1928)).

In no sense can the epithelial bodies be considered as representing vestigial remains of a milk dentition.

The concentric epithelial bodies described in *Canis*, *Cavia* and *Macropus* are not, I think, comparable with those of *Ornithorhynchus*, and in these forms they may well have the significance attributed to them by MARETT TIMS.

XI—THE DENTITION OF *ORNITHORHYNCHUS* IN RELATION TO BOLK'S THEORY

BOLK's dimery theory of the development and evolution of the mammalian teeth is of such outstanding importance that any observations made on mammalian tooth development should be considered in the light of this theory. Only by this means can sufficient evidence be accumulated to allow adequate criticism of his claims to be made.

BOLK founded his theory on four fundamental hypotheses:

1—Hypothesis of triconodonty. The primitive reptilian tooth is not a simple cone but is a triconodont tooth with a main central and smaller anterior and posterior cusps.

2—Hypothesis of dimery. Every mammalian tooth (with the exception of the elephants and the multituberculates) is the equivalent of two reptilian teeth. Thus a sextitubercular tooth is the fundamental mammalian structure.

3—Hypothesis of concentration. The anlagen of two reptilian teeth of the same "tooth family" are concentrated to give a single mammalian tooth. BOLK differentiates between concentration and concrescence; the latter implies the fusion of two separate and independent elements, evidence for which is lacking.

4—Hypothesis of equivalency. Every mammalian tooth is morphologically equivalent to every other and possesses the potentiality of developing a complicated crown pattern.

The obvious theoretical criticism of this theory is one which has been levelled against the concrescence theory, namely, that it is difficult to believe that the dental lamina can have a sufficiently long phylogenetic memory to enable it every now and again to bring to life, as it were, an extra reptilian tooth (whose existence would have been suppressed for many millions of years) in order to provide fresh cusps for the complicated mammalian molar.

To show the dimeric nature of a mammalian tooth BOLK brought forward evidence of the double nature of the enamel organ in the mammals. He said that the stellate reticulum is formed from two centres and that in early stages of development this can be recognized by the presence of an "enamel septum" of undifferentiated cells continuous with the stratum intermedium, which runs out to the surface of the enamel organ and completely subdivides it into two halves. This septum is of transitory nature and rapidly becomes merely a strand of cells. Again, BOLK claims that the dual nature of the enamel organ is shown by the presence of a second strand (the lateral enamel strand) connecting the enamel organ to the dental lamina; he says that this strand occurs universally in the mammals but is never seen in other animals. Its presence is

irregular in marsupials where some of the teeth are dimerous mammalian teeth but others are of monomerous reptilian nature (BOLK 1929).

With regard to the enamel septum, several observers have noticed a similar structure in the enamel organ but have described it as an enamel strand or cord, and deny that it is at any stage a complete septum. Moreover, MARCUS (1931) says that it arises relatively late, after the cells of the enamel pulp are differentiated, and therefore cannot be in the nature of a "reminiscence". Also, both he and WOERDEMAN (1919, 1921) have found a similar strand in the crocodiles. MARCUS says that such a strand is commonly seen in the marsupials, regularly so in the molar region, and that it shows the place where later the point of a cusp will be formed.

I have seen nothing resembling an "enamel septum" in any of the earlier stages of tooth development in *Ornithorhynchus* unless it is in the case of the upper tooth "y" of specimen H.J., where there is a faint indication of the sub-division of the enamel organ (fig. 45, Plate 38). Otherwise the only structures which might be related to the septum are the late developing ameloblastic strands described in specimens H.Q., Beta, and H.X.; these are always associated with the apices of cusps and they do not reach the surface of the enamel organ.

A lateral enamel strand is found in the earlier stages of development in connexion with most of the enamel organs of the cheek teeth of *Ornithorhynchus*; it is present in teeth "w", "x" and "y" of both jaws, though it is much more evident in the upper than in the lower teeth, and is only doubtfully present in the case of the lower tooth "y". In "x" and "y" the strand is best marked towards the posterior end of the enamel organ (fig. 39, Plate 37; figs. 45, 46, Plate 38). I think that what WILSON and HILL described as structural differentiations "of the nature of a series of invasions or deep indentations of the neck of the dental lamina, on its labial aspect, near the level of its continuity with the deep surface of the mouth-epithelium", and which they considered to be the forerunners of the epithelial nodules of later stages, in fact represent the mesodermal contents of BOLK's enamel tunnel which is bounded laterally by the lateral enamel strand. A comparison of fig. 46, Plate 38 with WILSON and HILL's fig. 1 leaves little doubt that the structure seen on the lateral side of the dental lamina is the same in both cases; fig. 46, Plate 38 shows a section through the posterior end of the enamel organ of the upper tooth "y", and, traced forwards, the lateral boundary of the indentation described by WILSON and HILL is continuous with a well-marked lateral enamel strand (fig. 45, Plate 38). This strand with its contained enamel tunnel is of almost constant occurrence in the mammals and cannot be related to the peculiar epithelial nodules found in the enamel organ of *Ornithorhynchus*.

What the significance of this lateral enamel strand may be is doubtful. In *Ornithorhynchus* at least it is not a mere crumpling or folding of the dental lamina or enamel organ as AHRENS (1913) and MARCUS (1931) believed, nor does it show any signs of representing an earlier dentition as KÜKENTHAL (1896) and ADLOFF (1916) have suggested.

GREGORY (1934, p. 192) points out that BOLK failed to explain the causal relations between the enamel niche and the enamel septum and merely assumed that they were parts of the same phenomenon. Certainly the fact that a niche is present and a septum is absent in *Ornithorhynchus* provides a striking example of the dissociation of these structures. Referring to the vascularity of the septum in *Phascolarctus*, GREGORY fails to see that BOLK has proved anything beyond the possibility of nutriment being carried via the septum to the crown of the tooth, and, since it is distributed on each side of the septum, two growth centres appear in the enamel organ. While GREGORY does not advance any explanation for the presence of the enamel niche, he suggests that it may represent a secondary vacuity developed in the dental lamina.

Finally, BOLK attempted to derive the diphyodont condition of the mammalian dentition from the polyphyodonty of the reptiles by showing that the latter possessed a "distichical" dentition and that in the mammals the "exostichos" (whose members always form first in the embryo) erupt first as the milk dentition, to be followed later by the endostichical elements which become the permanent teeth. The "exostichos" and "endostichos" are differentiated by the fact that the members of the former row develop as "parietal" enamel organs on the lateral side of the dental lamina, while the latter are formed at the extremity of the dental lamina as "terminal" enamel organs.

That the teeth of fish, amphibians and reptiles do alternate is well recognized, and such alternation appears to be an inherent and ancient property of the epidermis. It is present in the teeth and scales of modern sharks, it is seen in the teeth of the osteolepids, and again in the earliest tetrapods, the labyrinthodonts. PARRINGTON (1936) has recently shown that a "distichical" condition is present in the postcanine teeth of certain cynodont reptiles.

Whether these exostichical and endostichical rows become functionally independent and erupt with a long interval between them to give the "chorisstichic" dentition of the mammals, as BOLK claims, is without proof. Several authors have found no support for such a tooth row as BOLK's "odontostichos". It is claimed that the anlagen of all teeth at first arise at the free end of the dental lamina and later, with growth of the lamina, come to occupy a parietal position; that is to say, all teeth are "endostichical" and only secondarily become "exostichical" (WOERDEMAN, ADLOFF, MARCUS, MÜLLER and DRESSEL).

In *Ornithorhynchus* there is no evidence for the alternation of teeth; it has been shown that the enamel organs arise as "terminal" structures and, with further growth, they appear in a "parietal" position at a later stage. The enamel organ "w" is terminal, for example, in specimen X, but has become parietal in Delta: "x" is terminal in XXVIII B, but is parietal in H.N.

So far as *Ornithorhynchus* is concerned, the evidence does not uphold BOLK's view of the origin of the mammalian dentition. The dental lamina, however, is suppressed to such a degree and its products are so degenerate that it would be unwise to draw any far-reaching conclusions from the conditions present in the monotremes.

XII—SUMMARY AND CONCLUSIONS

1—The development of the dental lamina and of the enamel organs is described in a closely connected series of foetal specimens of *Ornithorhynchus*.

2—An incisor and canine region of the dental lamina is developed in both jaws; this is separated by a diastema from the more posterior portion which gives rise to those teeth which eventually erupt.

3—The incisor portion of the dental lamina of the upper jaw rapidly disappears, though the piece which occupies the position of a canine tooth is frequently retained until later stages.

4—The incisor region of the dental lamina of the lower jaw is present for a considerable time and shows evidence of the presence of five teeth in addition to the canine. They are all absorbed at a relatively early stage of development.

5—The full dental formula of *Ornithorhynchus* is: $i \frac{0}{8} c \frac{1}{1} pm \frac{2}{2} m \frac{3}{3}$.

6—Comparatively few of these developing teeth come to maturity and erupt. The "adult" dental formula is: $pm \frac{1}{0} m \frac{2}{3}$.

7—There is evidence of one milk tooth only in each jaw, this being in the premolar region.

8—Cusp development and dentine formation proceed from before backwards. This applies both to the individual teeth and to the tooth row as a whole, though the anterior part of a tooth is in a more advanced state of development than the posterior part of the tooth immediately in front of it.

9—The formation of enamel is independent of that of dentine and occurs at a much later stage. The enamel is degenerate.

10—The enamel organs become vascularized as development proceeds. This vascularity is probably associated with the formation of enamel. No enamel is deposited until the ingrowing capillaries lie in close contact with the stratum intermedium.

11—The epithelial nodules are divisible into two groups: (a) those which are constantly present inside the enamel organ and represent the detached apices of degenerate cusps, and (b) an adventitious group lying outside the enamel organs and only present in a few specimens; the significance of the latter group is obscure. The epithelial nodules do not represent a vestigial milk dentition.

12—Evidence is brought to show that even those teeth which erupt are in a very degenerate state. Therefore it is difficult to assess what phylogenetic value should be attached to them.

13—The morphology of the crowns of the teeth is described in various developmental stages. It is shown that "w" was originally a more complex tooth. In each of the larger teeth the two main cusps are always present (medial in the upper and lateral in

the lower jaw) and are the first to develop. Some of the lesser cusps developed on the cingulum appear to be fairly constant and may be of some morphological value.

14—The development of a complex root pattern is described.

15—The enamel organs are at first terminal and later acquire a parietal position so that there are no indications of a distichical arrangement of the teeth.

16—An enamel septum has not been found in any stage of development.

17—Apart from the presence of a lateral enamel strand in relation to the enamel organs of the posterior teeth, the significance of which is doubtful, there is no evidence of any fusion of enamel organs as might have been expected in a form like *Ornithorhynchus* on the basis of Bolk's dimery theory.

18—The only Mesozoic mammals which, from the point of view of tooth morphology, appear to be even remotely related to the monotremes are the Microleptidae. As, however, the teeth of *Ornithorhynchus* are so degenerate, it is unwise to attempt to base monotreme relationships on the present structure of their teeth.

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PLATE 32

FIG. 15—Specimen W. $\times 2.15$.

FIG. 16—Ventral aspect of specimen XXVIII B. $\times 1$.

FIG. 17—Specimen XXVIII B seen from the left side. $\times 1$.

FIG. 18—Ventral aspect of specimen H.X. after the removal of a block from the right side of the head and neck for sectioning. $\times 0.66$.

FIG. 19—Specimen H.X. seen from the right side. $\times 0.56$.

FIG. 20—Model of the dental laminae and the associated mouth epithelium of the left side of specimen W. Seen from the medial aspect. $\times 81$.



FIG. 15

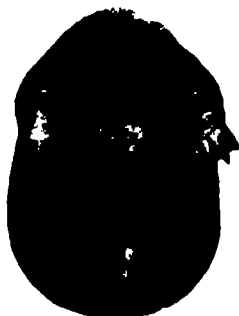


FIG. 16



FIG. 17



FIG. 18



FIG. 19

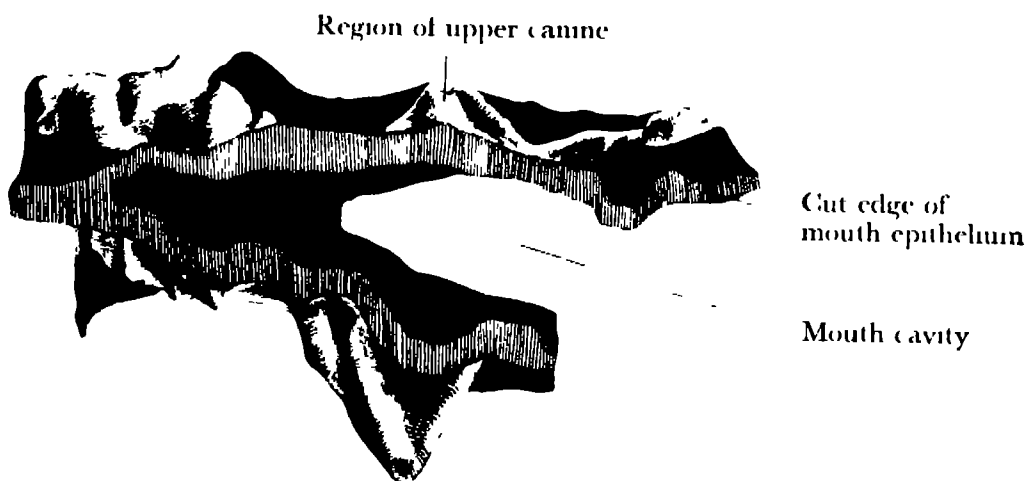


FIG. 20

PLATE 33

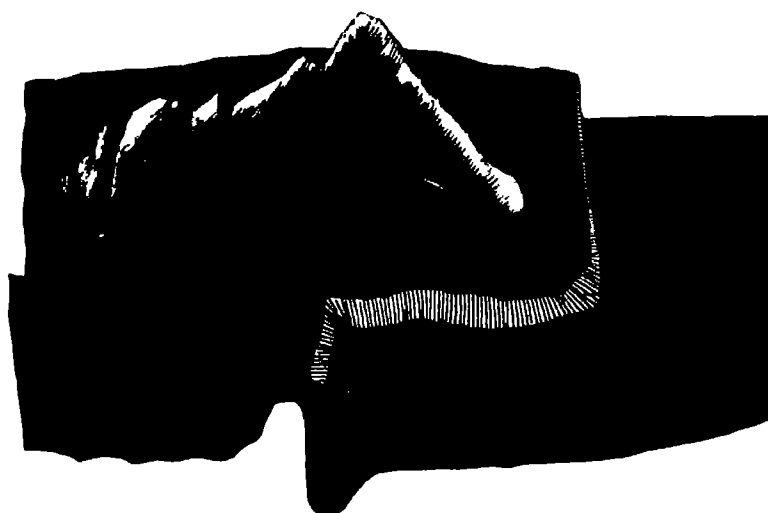
FIG. 21—Dental lamina of the left side of the upper jaw of specimen W, seen from above. The anterior end is to the right and the medial side is below. $\times 81$.

FIG. 22—Dental lamina of the left side of the lower jaw of specimen W, seen from below. The medial side is uppermost and the anterior end is to the right. $\times 81$.

FIG. 23—Specimen W, $\frac{3-1}{8}$. Transverse section showing condensation of the mesenchyme around the posterior end of the dental lamina of the upper jaw. The left side of the photograph is lateral. $\times 156$.



FIG 21



Buccal aspect of the
mouth epithelium of
the upper jaw

Cut edge of the
epithelium of
the lower jaw

FIG 22



FIG 23

PLATE 34

FIG. 24—Model of the dental lamina and mouth epithelium of the left side of the upper jaw of specimen X, seen from above. The lateral side is uppermost. $\times 37$.

FIG. 25—Model of the dental lamina of the left side of the lower jaw of specimen X. The medial side is uppermost. $\times 37$.

FIG. 28—Specimen X, $\frac{11-2}{8}$. Transverse section to show the vestigial tooth “*dv*” of the upper jaw lying at the junction of the lateral side of the neck of the dental lamina and the deep aspect of the mouth epithelium. $\times 200$.

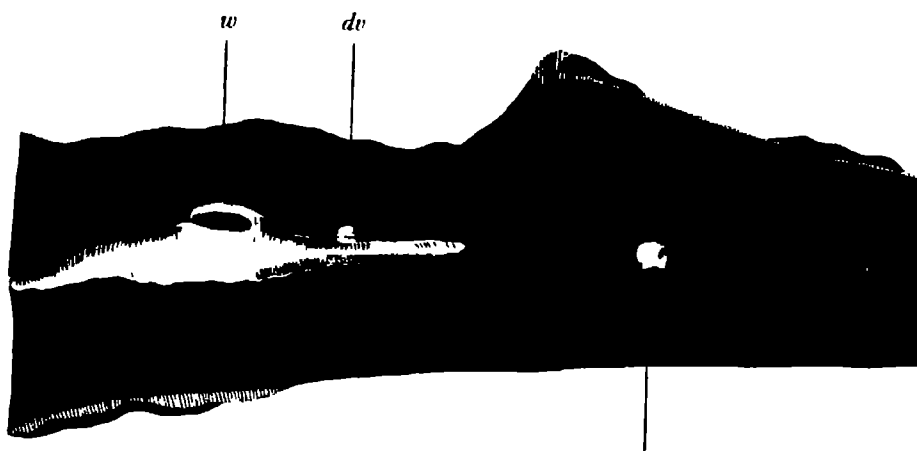


FIG. 24 Canine

Incisor and canine region

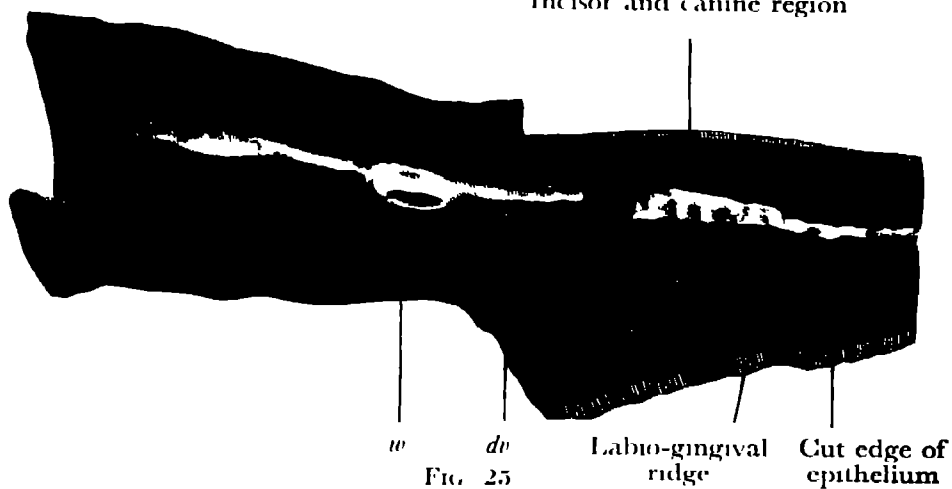


FIG. 25



FIG. 28

PLATE 35

FIG. 26—Model of the dental laminae and the mouth epithelium of the left side of specimen X. Seen from the lingual aspect. The cut edge of the mouth epithelium is hatched. $\times 37$.

FIG. 27—Model of the dental laminae and the mouth epithelium of the left side of specimen X. Seen from the buccal aspect. The cut edge of the mouth epithelium is hatched. $\times 37$.

FIG. 29—Model of the dental lamina of the right side of the lower jaw of specimen Delta, seen from the lateral aspect. $\times 33.5$.

FIG. 30—Model of the dental lamina of the right side of the lower jaw of specimen Delta, seen from below. The medial side is uppermost. $\times 33.5$.

FIG. 31—Model of the dental lamina of the right side of the upper jaw of specimen Delta, seen from the lateral aspect. $\times 35$.

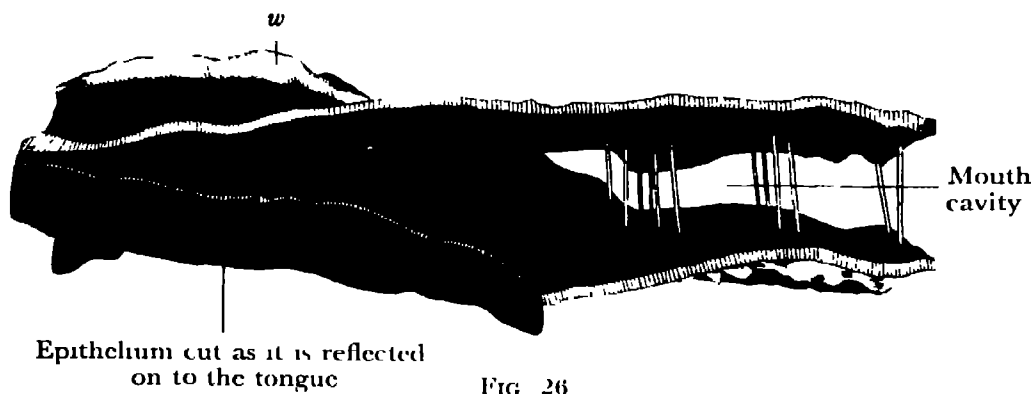


FIG. 26

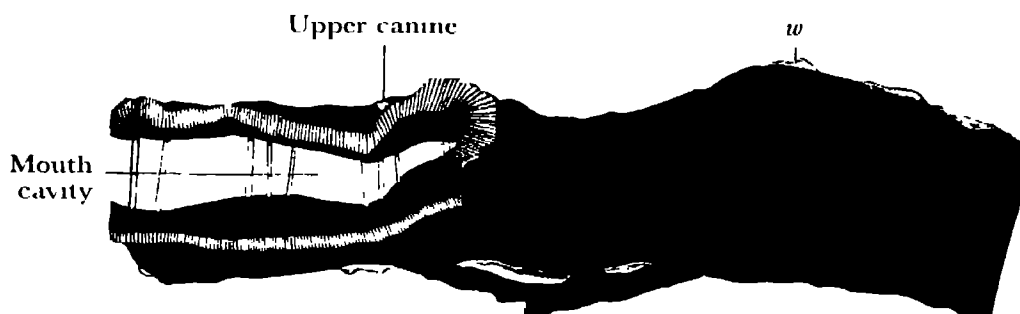


FIG. 27



FIG. 29

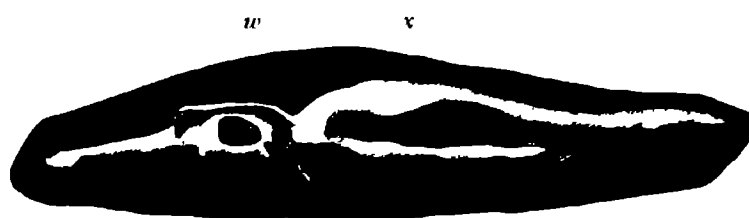


FIG. 30



FIG. 31

PLATE 36

FIG. 32—Model of the dental lamina of the right side of the upper jaw of specimen Delta, seen from above. The medial side is uppermost. $\times 33.7$.

FIG. 33—Model of the dental laminae of the right side of specimen XXVIII B, seen from the lateral aspect. The hatched surfaces indicate the cut edges of the mouth epithelium. $\times 21.3$.

FIG. 34—Model of the dental lamina of the right side of the upper jaw of specimen XXVIII B, seen from above. Note that "w" is a parietal enamel organ, "x" is becoming parietal (a residual dental lamina can be seen on its medial side), and "y" is terminal at this stage. The medial side is uppermost. $\times 21.3$.

FIG. 35—Model of the dental lamina of the right side of the lower jaw of specimen XXVIII B, seen from below. The lateral side is uppermost. $\times 21.3$.

FIG. 36—Specimen H.N. (Sag 50). The enamel organ and the degenerating dentinal shell of the lower tooth "w". $\times 77$.

FIG. 37—Specimen H.N. (Sag. 62). Epithelial nodule "dx₁" of the lower jaw. $\times 184$.

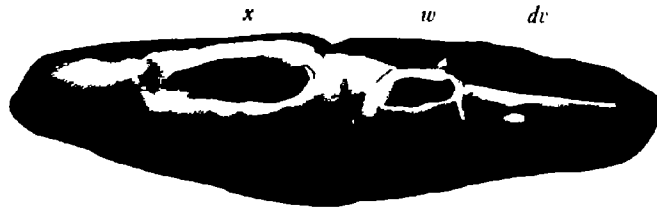


FIG. 32

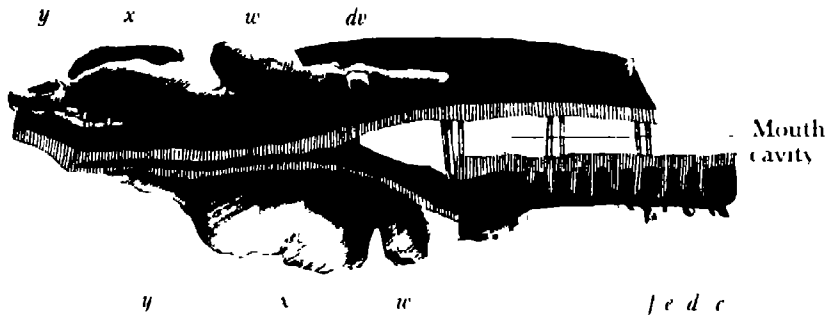


FIG. 33

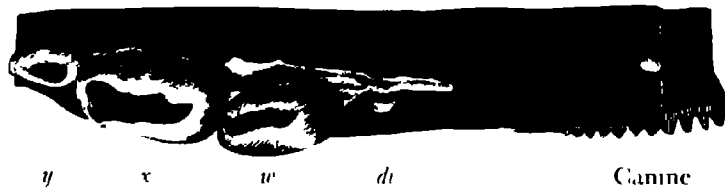


FIG. 34



FIG. 35

External enamel epithelium



FIG. 36

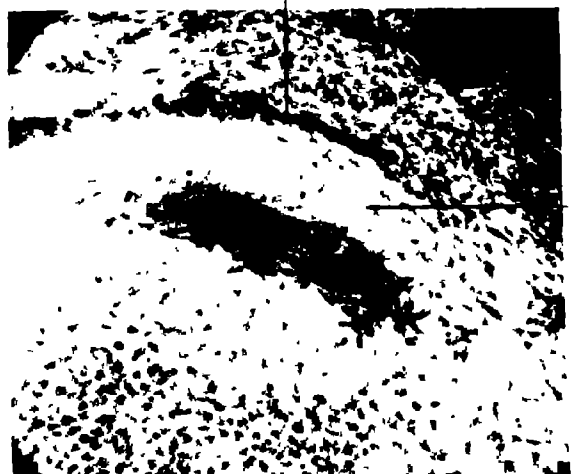


FIG. 37

Stellate reticulum

PLATE 37

FIG. 38—Specimen H.N. (Sag. 78). Epithelial nodule " dx_1 " of the upper jaw. $\times 184$.

FIG. 39—Specimen H.N. (Trans. 286). Section through the posterior part of the enamel organ of " x " of the upper jaw. A lateral enamel strand is seen in addition to the medial enamel strand (dental lamina), thus giving a double attachment of the enamel organ to the epithelium of the mouth. The lateral side is to the right in the photograph. $\times 46$.

FIG. 40—Specimen H.J. (Trans. 222). Section through the isolated piece of dental lamina of the right side of the upper jaw which occupies the position of a canine tooth. The lateral side is on the right of the photograph. $\times 148$.

FIG. 41—Specimen H.J. (Trans. 214). Section through " f " of the lower jaw showing the small, calcified dentinal papilla. The lateral side is on the right of the photograph. $\times 170$.

FIG. 42—Specimen H.J. (Trans. 274). Section through " dv " of the left side of the lower jaw. The dental lamina is seen on the medial side of the densely calcified nodule. $\times 148$.

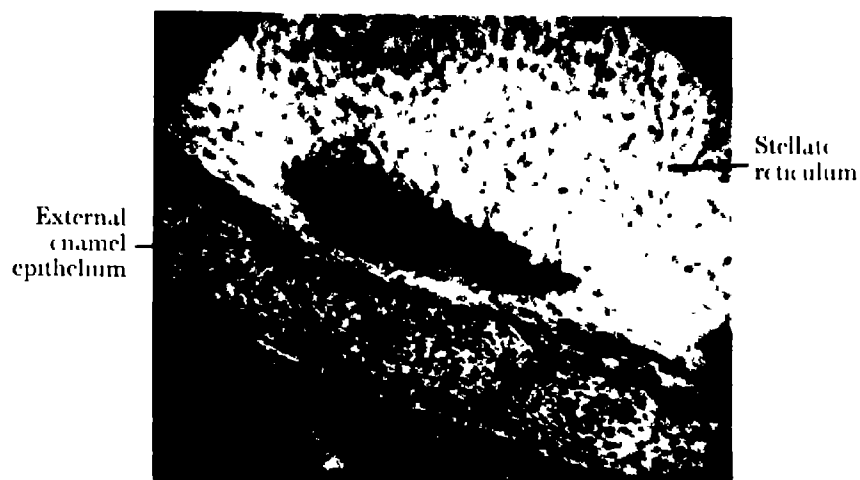


FIG. 38



FIG. 39



FIG. 40

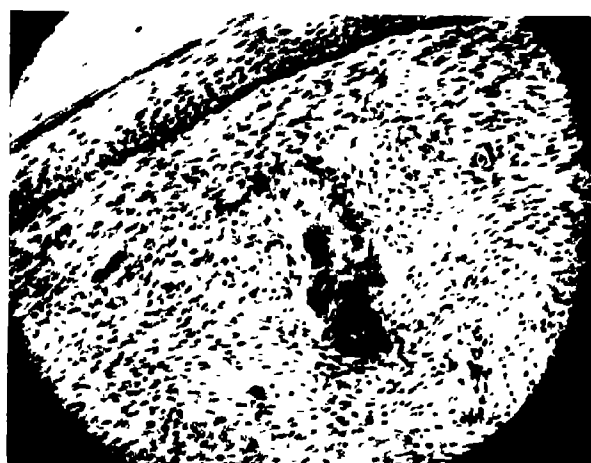


FIG. 41



FIG. 42

PLATE 38

FIG. 43—Specimen H.J. (Trans. 282). Section through the left lower “*w*”. The degenerated core of dentine is completely surrounded by a capsule of cells derived from the enamel organ. The dental lamina is seen on the medial side of “*w*”. × 93.

FIG. 44—Specimen H.J. (Trans. 385). Section through the enamel organ and the postero-medial cusp of the right upper “*x*” to show an early stage in the formation of the epithelial nodule “*dx₂*”. Fragmentary remains of the lateral enamel strand are seen. × 44.

FIG. 45—Specimen H.J. (Trans. 408). Section through the enamel organ of the right upper “*y*”. A lateral enamel strand is present in addition to the dental lamina. A blood vessel is seen entering the stellate reticulum. × 39.

FIG. 46—Specimen H.J. (Trans. 414). Section through the posterior part of the enamel organ of the left upper “*y*”. The lateral enamel strand is seen to bound the space which WILSON and HILL described as an indentation of the lateral side of the neck of the dental lamina (compare WILSON and HILL’s fig. 1). × 42.



FIG. 44



FIG. 46



FIG. 43



FIG. 45

PLATE 39

FIG. 47—Model of tooth “x” of the left side of the lower jaw of specimen H.P. seen from the medial aspect. Cusps 1 and 2 are starting to develop on the medial cingulum (compare fig. 68, Plate 42; fig. 84, Plate 45; and fig. 12). × 31.

FIG. 48—Model of tooth “x” of the left side of the lower jaw of specimen H.P. seen from the lateral aspect. × 31.

FIG. 49—Occlusal surface of the left lower “x” of specimen H.P. The medial side of the tooth is uppermost. × 31.

FIG. 50—Model of tooth “y” of the left side of the lower jaw of specimen H.P. seen from the medial aspect. × 31.

FIG. 51—Model of tooth “y” of the left side of the lower jaw of specimen H.P. seen from the lateral aspect. × 31.

FIG. 52—Occlusal surface of the left lower “y” of specimen H.P. The medial side of the tooth is uppermost. × 31.

FIG. 53—Lateral aspect of tooth “x” of the left side of the upper jaw of specimen H.P. × 31.

FIG. 54—Medial aspect of tooth “x” of the left side of the upper jaw of specimen H.P. × 31.

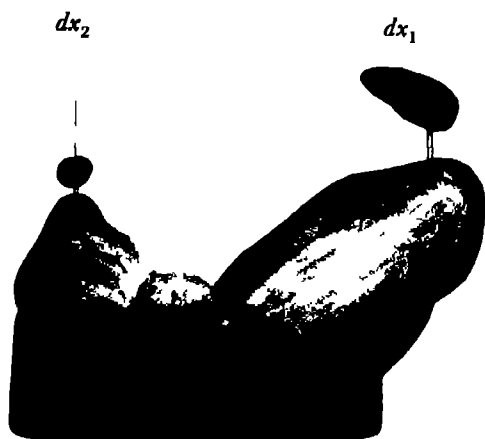


FIG. 47

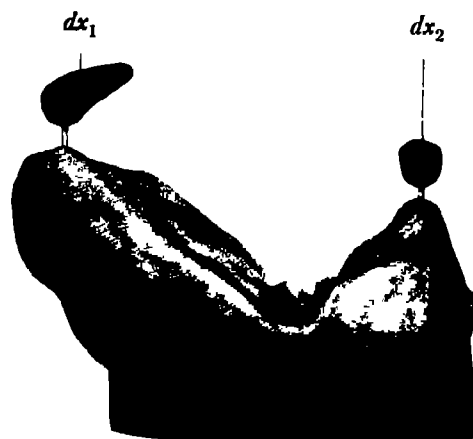


FIG. 48

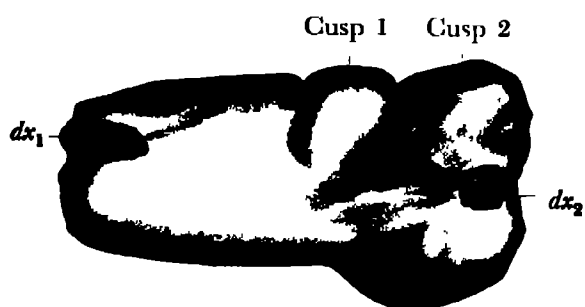


FIG 49



FIG 50



FIG 51

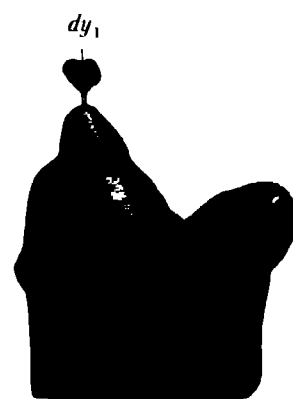


FIG 52

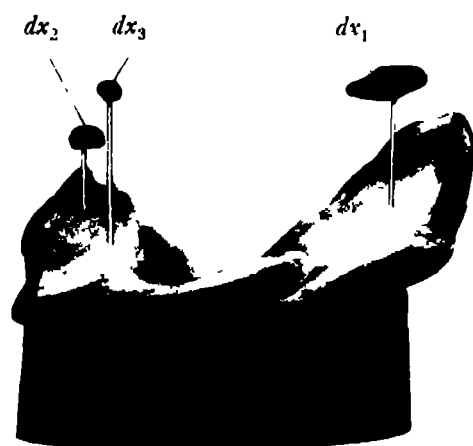


FIG 53

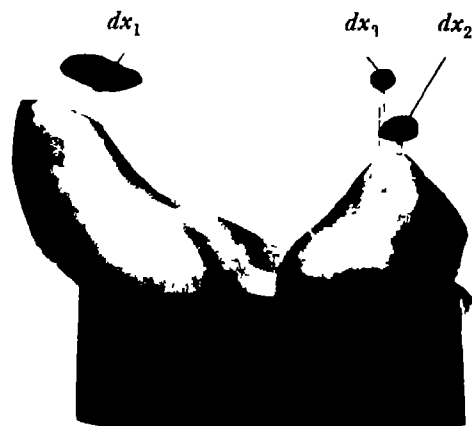


FIG 54

PLATE 40

FIG. 55—Occlusal surface of the left upper “*x*” of specimen H.P. The lateral border of the tooth is uppermost. $\times 31$.

FIG. 56—Lateral aspect of tooth “*y*” of the left side of the upper jaw of specimen H.P. $\times 31$.

FIG. 57—Medial aspect of tooth “*y*” of the left side of the upper jaw of specimen H.P. $\times 31$.

FIG. 58—Occlusal surface of the left upper “*y*” of specimen H.P. The lateral border of the tooth is uppermost $\times 31$.

FIG. 59—Specimen H.P. Occlusal surfaces of teeth “*x*” and “*y*” of both jaws to show the amount of dentine formation at this stage. Areas covered by dentine are stippled, the rest of the crowns of the teeth being uncalcified. The epithelial nodules are shown in black. $\times 24$.

FIG. 60—Specimen H.P. (Sag. 86). The tip of the calcified cusp of the lower tooth “*w*” is cut transversely, surrounded by its stellate reticulum. If this is compared with fig. 65, Plate 41, it is possible to imagine how separation and further degeneration of the apex of a cusp might lead to the appearance of an epithelial body. $\times 43$.

FIG. 61—Specimen H.P. (Sag. 124). Showing the postero-lateral cusp of the lower “*x*” with its degenerated cap of dentine and the associated epithelial nodule “*dx₂*”. The latter is in an early stage of formation and is still deeply embedded in the stellate reticulum. $\times 74$.

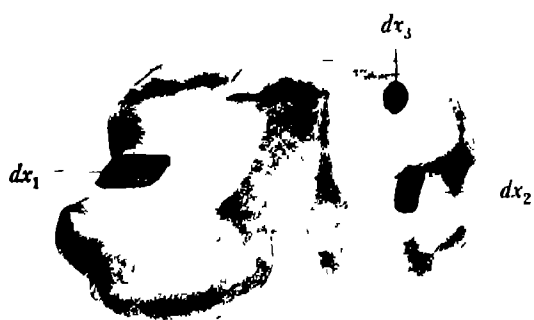


FIG 55



FIG 56



FIG 57

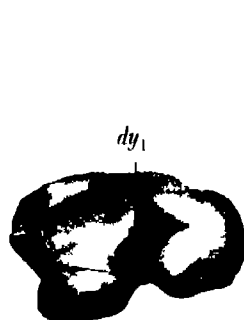


FIG 58

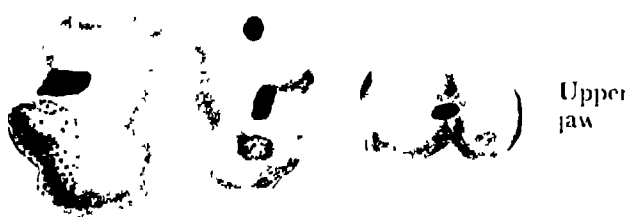


FIG 59



FIG 60

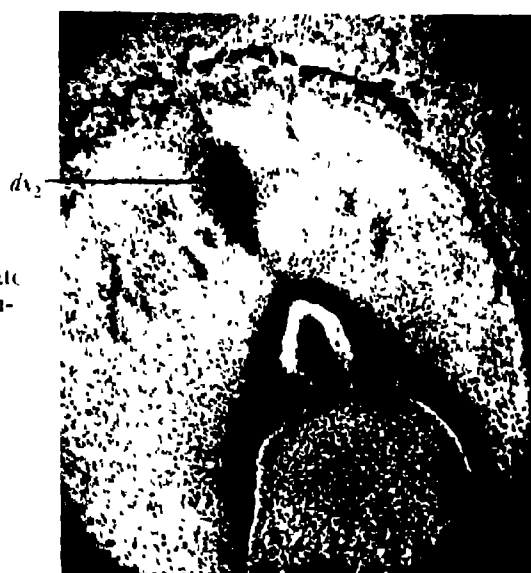


FIG. 61

PLATE 41

FIG. 62—Specimen H.P. (Sag. 130). The epithelial body " dx_1 " which is related to the antero-medial cusp of the upper tooth " x ". $\times 162$.

FIG. 63—Specimen H.P. (Sag. 128). Epithelial nodule " dx_2 " which is related to the postero-medial cusp of the upper tooth " x ". $\times 184$.

FIG. 64—Specimen H.P. (Sag. 140). Epithelial nodule " dx_3 ". It lies outside the enamel organ of the upper tooth " x ", close to the mouth epithelium. $\times 44$.

FIG. 65—Specimen H.Q. (Trans. 448). Epithelial nodule " dx_1 " of the lower jaw. The external enamel epithelium is seen over the superficial surface of the nodule. $\times 206$.



External
enamel
epi-
thelium
Stellate
reticulum
Mouth
epi-
thelium

FIG. 62



Stellate
reticulum

FIG. 65



Stellate
reticulum
Epi-
thelium
of upper
jaw
External
enamel
epi-
thelium

FIG. 63



Stellate
reti-
culum

dx_3

FIG. 64

PLATE 42

FIG. 66—Specimen H.Q. (Trans. 504). Epithelial nodule " dx_2 " of the lower jaw. It is cornified and is still related to the tip of the postero-lateral cusp of " x " by a strand of cells. $\times 71$.

FIG. 67—Model of the right lower " x " of specimen Beta, seen from the lateral aspect. $\times 17.5$.

FIG. 68—Medial aspect of the right lower " x " of specimen Beta. Cusps 1, 2, 3 and 4 are present in addition to the two main cusps (compare fig. 47, Plate 39; fig. 84, Plate 45; fig. 12). $\times 17.5$.

FIG. 69—Occlusal surface of the right lower " x " of specimen Beta. The lateral border of the tooth is uppermost. $\times 17.5$.

FIG. 70—Lateral aspect of the right lower " y " of specimen Beta. The adventitious epithelial nodule (" dy_1 " of WILSON and HILL) is shown as well as the two constant nodules. $\times 17.5$.

FIG. 71—Medial aspect of the right lower " y " of specimen Beta. $\times 17.5$.

FIG. 72—Occlusal surface of the right lower " y " of specimen Beta. The lateral border of the tooth is uppermost. $\times 17.5$.

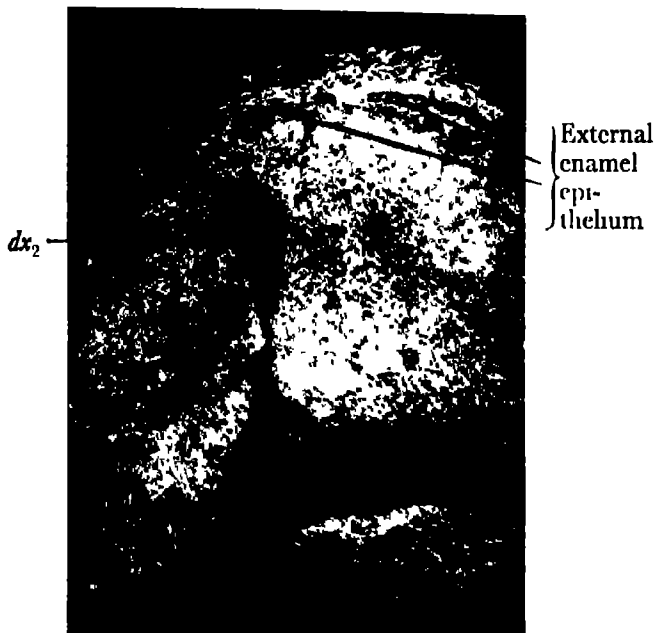


FIG 66



FIG. 72



FIG. 69

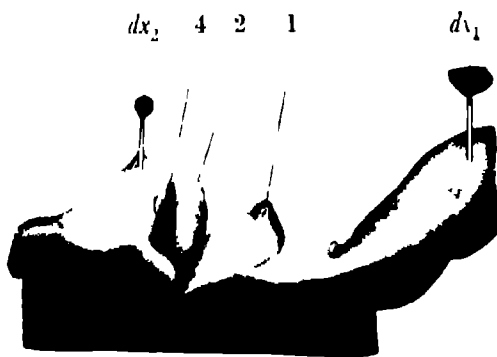


FIG. 67

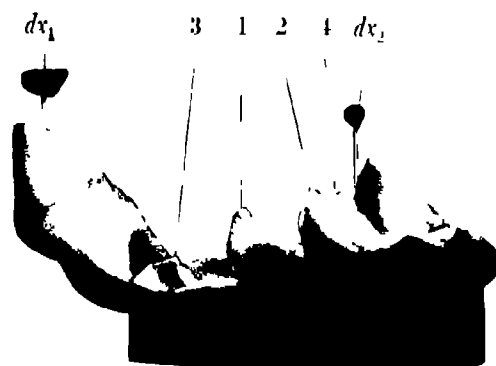


FIG 68

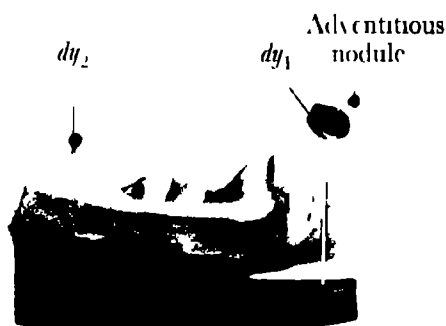


FIG 70

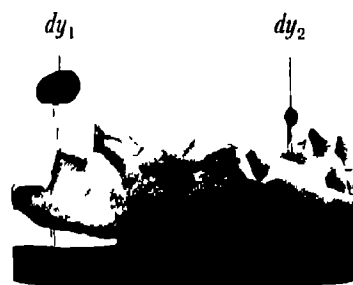


FIG 71

PLATE 43

FIG. 73—Lateral aspect of the right upper “x” of specimen Beta. In contrast with the lower teeth of this specimen the cingulum has not yet differentiated any cusps. $\times 17.5$.

FIG. 74—Medial aspect of the right upper “x” of specimen Beta. $\times 17.5$.

FIG. 75—Occlusal surface of the right upper “x” of specimen Beta. The medial border of the tooth is uppermost. The close relation of the epithelial nodules to the main cusps is clear. $\times 17.5$.

FIG. 76—Lateral aspect of the right upper “y” of specimen Beta. $\times 20$.

FIG. 77—Medial aspect of the right upper “y” of specimen Beta. $\times 20$.

FIG. 78—Occlusal surface of the right upper “y” of specimen Beta. The epithelial nodule “ dy_1 ” is seen to have two or three subsidiary bodies related to it medially. The adventitious toothlet (WILSON and HILL’s “ dy_2 ”) lies directly posterior to “ dy_1 ”. $\times 20$.

FIG. 79—Specimen Beta. View of the occlusal surfaces of teeth “x” and “y” of both jaws to show the areas over which dentine has been developed; these areas are stippled. No enamel is present and the greater parts of the teeth are not yet calcified. Compare with fig. 59, Plate 40. $\times 12$. N.B. A small stippled area should be shown over the most anterior of the cuspules on the medial border of the lower tooth “y”; it has been inadvertently omitted in the drawing.

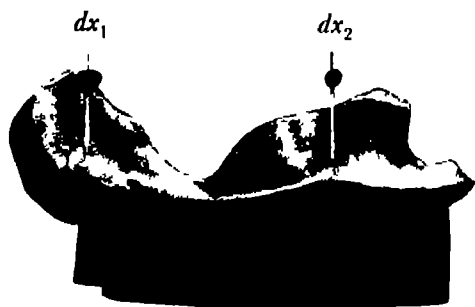


FIG. 73

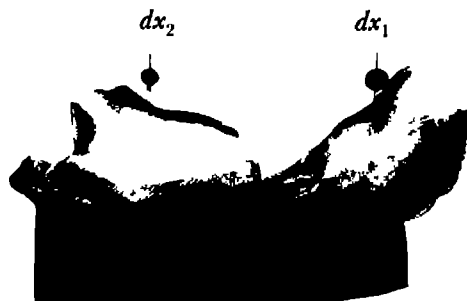


FIG. 74



FIG. 75

Adventitious toothlet



FIG. 78

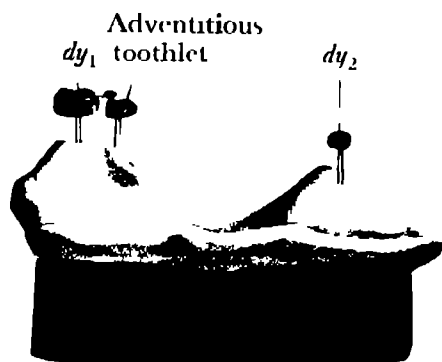


FIG. 76

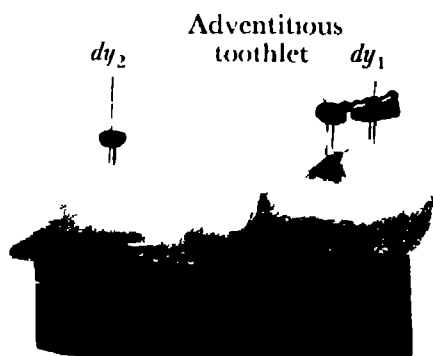


FIG. 77



FIG. 79

PLATE 44

FIG. 80—Specimen H.P. (Sag. 117). Epithelial body which might be compared with the adventitious toothlet in specimen Beta. It is here continuous with the mouth epithelium and lies outside the enamel organ. $\times 80$.

FIG. 81—Specimen H.X. (Sag. 125). A low power view showing the enamel organs in both jaws. The vascularity of the enamel organs is striking. In addition to the dentine there is a layer of enamel (darkly staining) over the cusps of the teeth. Epithelial nodules " dx_2 " and " dy_1 " of the lower jaw are apparent. $\times 8.75$.



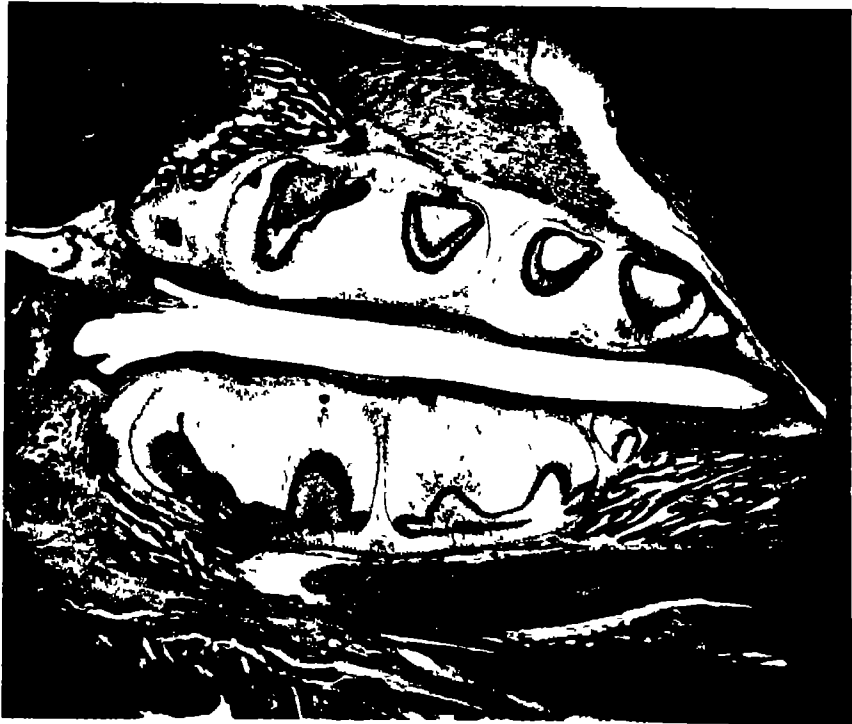
FIG. 80

Upper jaw

w

x

y



x

y

z

Lower jaw

FIG. 81

PLATE 45

FIG. 82—Specimen H.X. Drawing of wax models showing the occlusal surfaces of the lower teeth of the right side. $\times 15.5$. In this, and succeeding figures (up to fig. 87, Plate 46), the stippled areas indicate the amount of enamel that is present.

FIG. 83—Lateral aspect of the right lower teeth of specimen H.X. $\times 15.5$.

FIG. 84—Medial aspect of the right lower teeth of specimen H.X. In tooth "x" the additional cusps 1, 2 and 4 are shown (compare fig. 47, Plate 39; fig. 68, Plate 42; fig. 12). $\times 15.5$.

FIG. 85—Occlusal surfaces of the right upper teeth of specimen H.X. $\times 15.5$.



FIG. 82

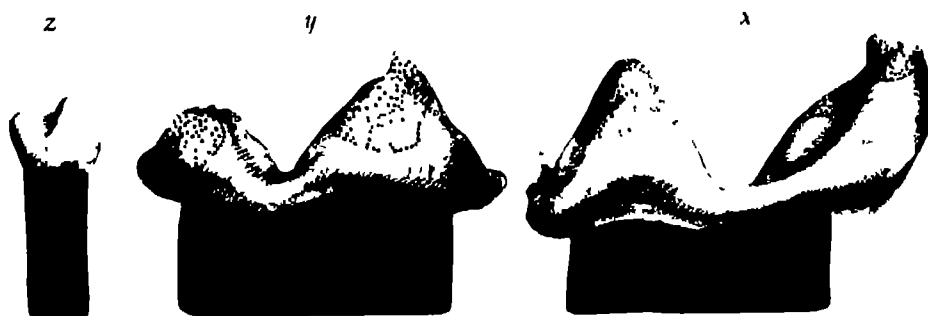


FIG. 83

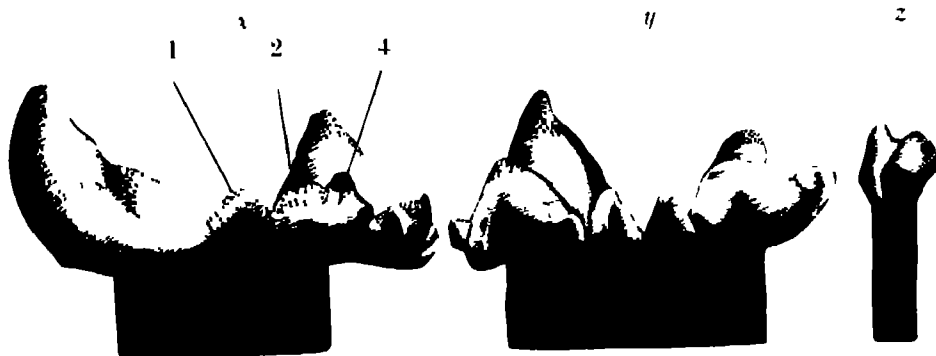


FIG. 84



FIG. 85

PLATE 46

FIG. 86—Lateral aspect of the right upper teeth of specimen H.X. The two pairs of cingular cusps, each pair related to one of the main medial cusps of tooth "x", are shown (compare fig. 12). $\times 15.5$.

FIG. 87—Medial aspect of the right upper teeth of specimen H.X. $\times 15.5$.

FIG. 88—Specimen H.X. Drawing of model showing the lateral aspect of the enamel organs of the lower teeth of the right side. Nodules " dy_1 " and " dx " may be seen. $\times 10.7$.

FIG. 89—Specimen H.X. Medial aspect of a model of the enamel organs of the right upper teeth. Nodules " dy_1 ", " dy_2 " and " dx_2 " may be seen. $\times 10.7$.

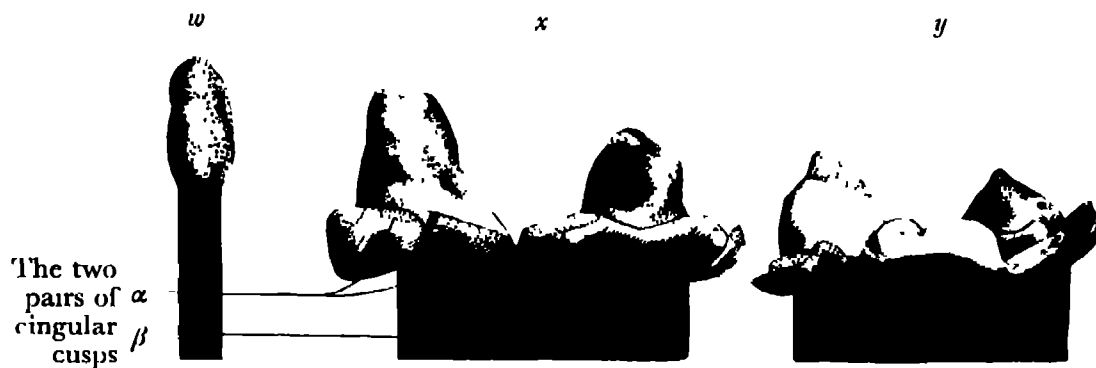


FIG. 86



FIG. 87



FIG. 88



FIG. 89

PLATE 47

FIG. 90—Specimen H.X. View of the superficial aspect of the enamel organs of the right lower teeth from above. $\times 10.7$. In these models "windows" have been cut in the enamel organs to show the epithelial nodules which are embedded in them. They are all situated close to the external enamel epithelium at this stage and in figs. 88 and 89 one or two can be seen projecting apparently beyond the general level of the surface of the enamel organ: they would not be seen in this way if the enamel organ had not been dissected to expose them.

FIG. 91—Specimen H.X. The enamel organs of the right upper teeth seen from below. The prominences caused by the large medial cusps are obvious. $\times 10.7$.

FIG. 92—Specimen H.X. (Sag. 98). Degenerated remains of the lower tooth "*w*"; there is a dentinal nodule with a cap of enamel surmounted by the remains of the enamel organ. $\times 98$.

FIG. 93—Specimen H.X. (Sag. 104). The antero-lateral cusp of the lower "*x*" and the epithelial nodule "*dx₁*". The irregularity of the surface of the enamel over the cusp, the degenerated structure of the nodule, and the continuity of the external enamel epithelium over the surface of the nodule are all to be noticed. $\times 46$.

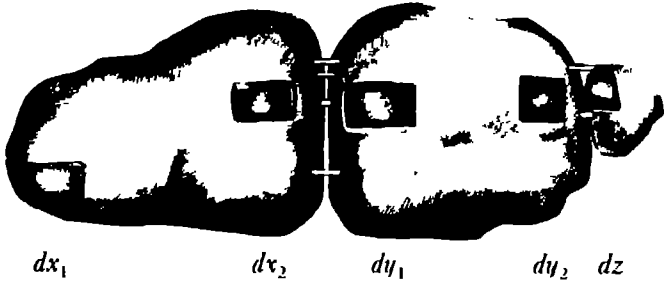


FIG. 90

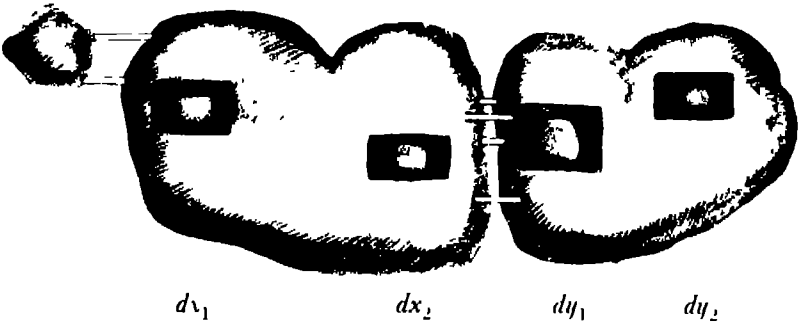


FIG. 91



FIG. 92



FIG. 93

PLATE 48

FIG. 94—Specimen H.X. (Sag. 128). To show the epithelial body " dy_1 " of the lower jaw lying inside the external enamel epithelium, and the nodules of enamel in the stellate reticulum between it and the cusp (the cusp is not shown in the picture). $\times 97$.

FIG. 95—Specimen H.X. (Sag. 105). To show the presence of an epithelial nodule in an early stage of differentiation overlying the anterior cusp of the medial cingulum of the lower tooth " y ". $\times 42$.

FIG. 96—Specimen H.X. (Sag. 109). An epithelial body " dw " is seen in relation to the apex of the upper " w ". $\times 170$.

FIG. 97—Specimen H.X. (Sag. 127). A section through the postero-medial cusp of the upper tooth " y ". In the neighbourhood of the degenerate and bent portion of the apex of the cusp are seen scattered nodules of enamel and an ameloblastic strand. The epithelial body " dy_2 " is just shaved. $\times 81$.

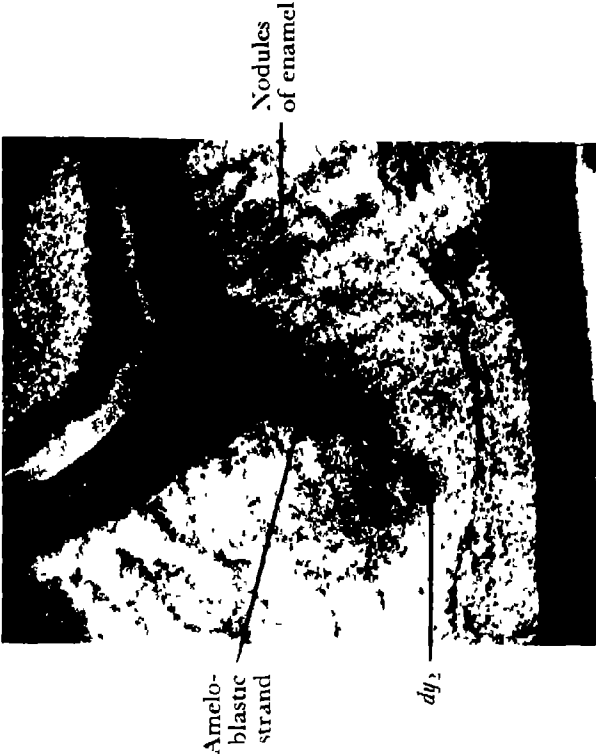
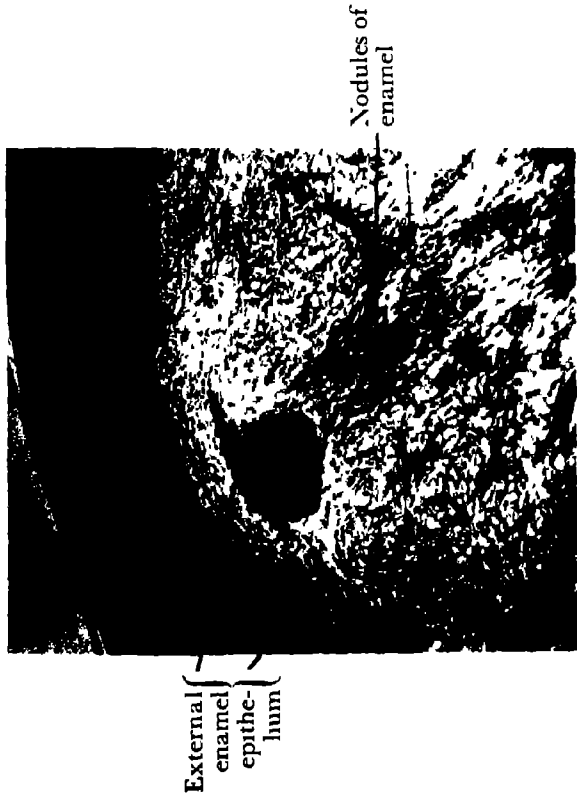


PLATE 49

FIG. 98—Specimen H.X. (Sag. 132). Section through the antero-medial cusp of the upper “y” to show the great vascularity of the enamel organ. The vessels are seen to reach the stratum intermedium and in the area where this occurs it will be seen that a layer of enamel has been deposited. $\times 45$.

FIG. 99—Specimen H.X. (Sag. 141). A section through another part of the same tooth as that shown in fig. 98. The blood vessels lying in the enamel organ are seen to stop abruptly some distance from the stratum intermedium so that there is a thick avascular layer of stellate reticulum interposed. No calcification has yet commenced in this area. $\times 45$.



FIG 98



FIG. 99

STUDIES ON THE ONYCHOPHORA

IV.—THE PASSAGE OF SPERMATOOZOA INTO THE OVARY IN
PERIPATOPSIS AND THE EARLY DEVELOPMENT OF THE OVA*

By S. M. MANTON, M.A., Sc.D.

(Mrs J. P. Harding)

*Fellow of Girton College**From the Zoological Laboratory, Cambridge*

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[PLATES 50 and 51]

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THE OVARY AND THE EARLY DEVELOP- MENT OF THE OVA	428	DISCUSSION	437
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THE ENTRY OF SPERMATOOZOA INTO THE FEMALE *PERIPATOPSIS*

The males of some species of Onychophora form large spermatophores with a definite shape, and it is supposed that these spermatophores are deposited in the female genital opening. These species also possess paired receptacula seminis which contain most of the spermatozoa that may be found in the female genital tract. Near the receptaculum seminis there may be a ciliated funnel communicating with the body cavity (*Peripatidae*).

In other species, notably most of the *Peripatopsidae*, the male deposits small spermatophores anywhere on the body surface of the female. The receptacula seminis are almost or completely absent in *Opisthopatus* and *Peripatopsis*, and spermatozoa are found in the lumen of the ovarian tubes. No open communication exists between the female genital tract and the haemocoel.

The manner of entry of the spermatozoa into the female genital tract from the small spermatophores attached to the body surface has hitherto been unknown. As the

* Studies on the Onychophora. I, Digestive Enzymes of *Peripatopsis*, by N. G. Heatley, *J. Exp. Biol.* 13, 329-43; II, The Feeding, Digestion, Excretion, and Food Storage of *Peripatopsis*, by S. M. Manton and N. G. Heatley, *Philos. Trans. B*, 227, 411-64; III, The Control of Water loss in *Peripatopsis*, by S. M. Manton and J. A. Ramsay, *J. Exp. Biol.* 14, 470-2.

cuticle is dry, it is impossible for the spermatozoa to reach the external genital opening directly from the spermatophores, and the problem remains as to "how these little packets of spermatozoa get into the vagina, and then up the uteri, which are always full of embryos" (SEDGWICK 1885). WILLEY (1898) states that spermatozoa in *P. capensis* are "probably injected into the body-cavity through the body-wall by the process described by Whitman as *hypodermic injection*. In the case of leeches...this has been satisfactorily observed." This suggestion has been accepted as a fact by some later writers, and ZACHER (1933) remarks "Der Mechanismus der Begattung ist noch nicht aufgeklärt...bei *Peripatopsis capensis*...die Spermatophoren sollen die Körperwand durchdringen und in die Leibeshöhle gelangen", but no facts support these speculations.

SEDGWICK (1909) discusses the problem more fully. "It has been suggested that the spermatozoa make their way from the adherent spermatophore through the body wall into the body, and so by traversing the tissues reach the ovary; but having regard to the thickness and toughness of the skin and the absence of any cutaneous secretion capable of dissolving the coat of the spermatophore, it seems unlikely that this should occur. We therefore venture to make the suggestion, though we cannot offer any facts in support of it, except the swallowing of the cuticle..., that the creatures lick the spermatophores off their bodies or otherwise devour them, and that the spermatozoa are set free in the stomach, and make their way through its soft walls and through the body cavity to the ovary or receptaculum seminis."

Material for the solution of this problem has been provided by specimens of several species of *Peripatopsis* which have been kept in captivity during the last four years, and which have been killed at various seasons, primarily for other purposes (MANTON and HEATLEY 1937).

ZACHER's suggestion can be eliminated. Many spermatophores have been observed during the periods between ecdyses, and they are never detached from the body wall nor do they penetrate through it.

It is equally certain that spermatophores are not licked off the body by the female, but it is clear that spermatophores can reach the intestinal cavity when the cuticle is eaten after ecdysis. Numerous sections of intestines (prepared for other purposes) give no evidence of sperm gaining entry to the body cavity via the intestine. No sperm has been found in the intestinal lumen or in its walls; and it is probable that the protoplasmic contents, if any, of a spermatophore in the intestine are digested by the intestinal juices which are suited to a carnivorous diet (MANTON and HEATLEY 1937). Further, some spermatophores on cast cuticles are devoid of spermatozoa.

The occurrence of active spermatozoa, not only in the ovary, but in the haemocoel outside the egg follicles, was recorded by MOSELEY (1874). He remarks "they probably commonly escape amongst the viscera", and presumably he visualized the sperm escaping from the ovary to the haemocoel.

Details of the passage of the spermatozoa into the body have been followed in *P. sedgwicki*, but the process is probably similar in other species of the genus. Freshly

deposited spermatophores have been watched until they are removed with the cuticle at ecdysis, and sections have been prepared of the body wall and attached spermatophore at known intervals after deposition. Duboscq Brazil proved to be a very satisfactory fixative, but embedding had to be done immediately after fixation in order to avoid hardening. Mallory's triple stain and iron haematoxylin were the most useful stains.

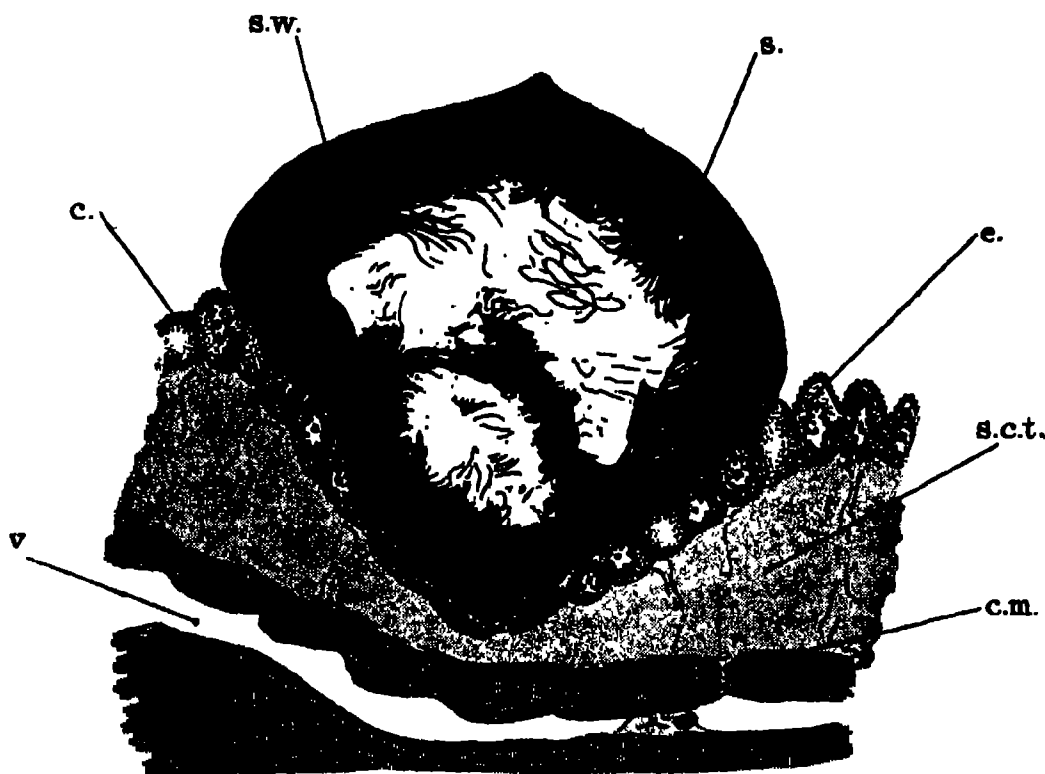


FIG. 1.—Vertical section of a spermatophore attached to the body wall 48 hr. after deposition. The integument in this and the following figures is shown in black. ($\times 679$ approx.)

The process of copulation in *Peripatopsis* will be described in a general account of the life-history which is in course of preparation. Each spermatophore is a closed capsule filled with sperm, and is placed anywhere on the body surface of the female. The spermatophores are variable in size and shape, they are usually irregularly rounded, and the diameter is often about 200μ . When freshly deposited they appear white and glistening, and adhere closely to the integument either between or over the primary papillae of the body. A small spermatophore fitting in between two papillae is shown in fig. 1. The wall of the spermatophore is tough and resistant and is evidently waterproof. It is at first fairly even in thickness, and in section appears to be composed of large granules stuck together. The wall stains red with Mallory's triple stain. Within it

the spermatozoa are tightly packed. No change can be seen in either spermatophore or body wall for several days.

From the third to the fifth day after deposition of a spermatophore the thick subcutaneous connective tissue layer below it becomes invaded by cells which are either leucocytes from the haemocoel or cells derived from the intermuscular connective tissue. Normally very few cells can be seen in the outer connective tissue. These

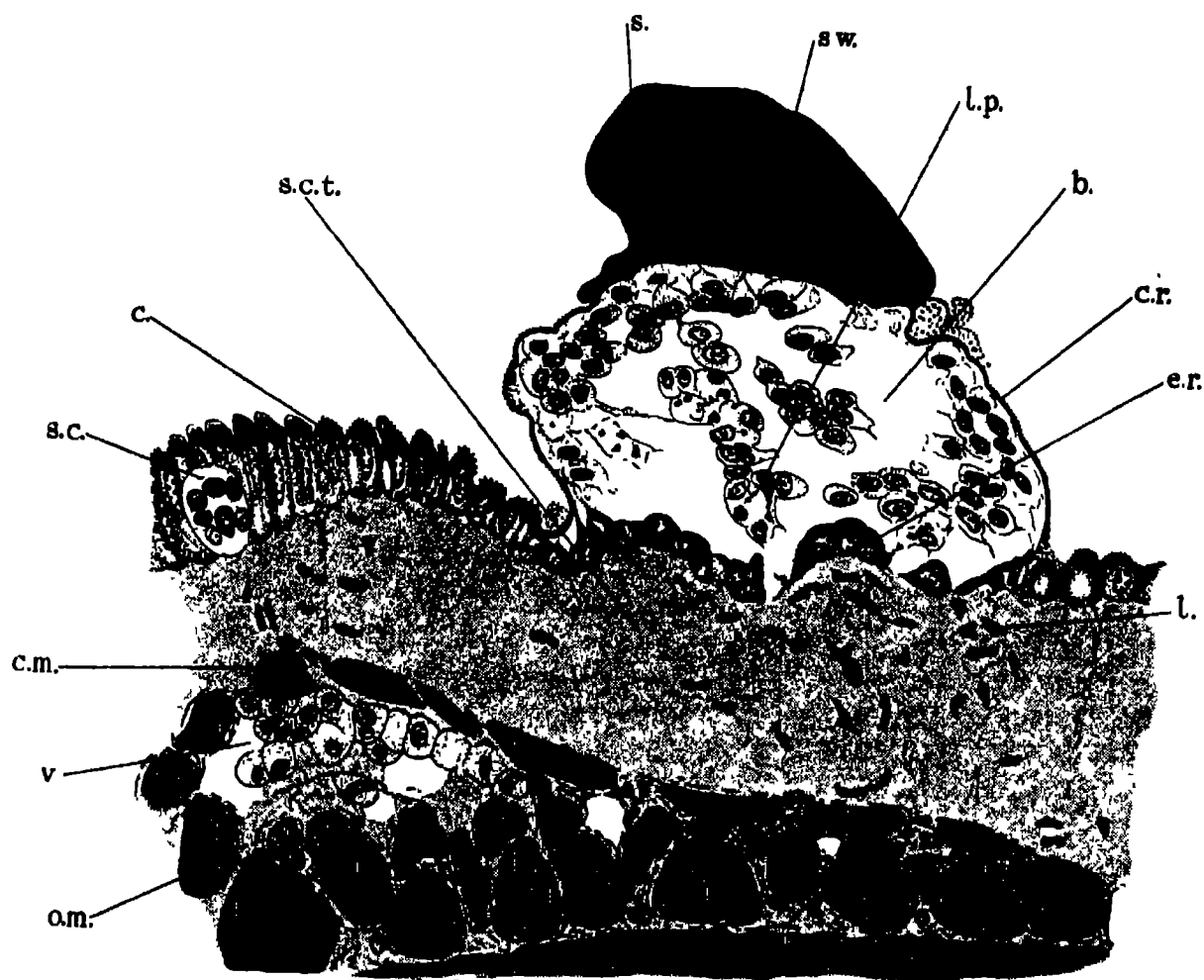


FIG. 2—Vertical section of spermatophore on the body wall seven days after deposition.
For description see text. $\times 324$ approx.

leucocytes break through the ectoderm below the spermatophore and accumulate between the ectoderm and the overlying cuticle, which is thereby pushed away from the body. Some ectodermal cells are partially ingested, and within many leucocytes there are patches of pigment granules from the ectoderm cells (fig. 2, *l.p.*). Gaps are thus left in the ectodermal epithelium which communicate with a large blister-like swelling lying below the cuticle and adherent spermatophore. This condition is reached

seven days after deposition (fig. 2). Externally the elevated spermatophore is still white and glistening.

A short time after the formation of this blister the cuticle and lower wall of the spermatophore become perforated. Whether the cuticle is burst mechanically by the distension of the swelling, or whether the leucocytes directly cause its breakdown is unknown. Spermatozoa then leave the spermatophore and swim actively into the swelling. They pass through the gaps in the ectodermal epithelium and wriggle in between the fibres of the outer layer of connective tissue. From here they spread sideways and inwards reaching the vascular channels of the muscular layers. A small group of spermatozoa in the muscle layer near an empty spermatophore is shown in fig. 4. These spermatozoa are overlying but not penetrating the muscles, and are situated in the loose connective tissue sheaths of the muscle fibres. No sperms were ever found in the tissues near freshly deposited spermatophores full of sperm. Once the integument is perforated the passage of the spermatozoa into the body is rapid, and is completed possibly in an hour or so as it is in some leeches.

After the spermatozoa have left the spermatophore its walls collapse and form a disk-shaped covering to the swelling. Within the latter the leucocytes form a degenerating mass, and those in the connective tissue layer become fewer. Such a stage is shown in fig. 3. The exact age of this very large spermatophore is unknown, but it was more than ten days old (only half of it is shown). Two spermatozoa (*s.*) still remain in the swelling and one in the ectoderm in the section figured. As soon as the spermatozoa pass into the body the spermatophore loses its white appearance and becomes dull and grey.

The degeneration of the leucocytes is followed by the sinking down of the ruptured cuticle and the reorganization of the ectodermal epithelium. The spermatophore wall remains adherent, and thus at all times forms an effective covering of the wound produced by the entry of the sperm. The reorganized ectoderm may be irregular, but it is continuous, and in due course forms a new cuticle below the perforated one. This new cuticle is continuous with that formed elsewhere. Such a stage is shown in fig. 5. The gaps in the old cuticle below the spermatophore wall are clearly seen, while elsewhere the cuticle is double. This animal was about to cast its skin. A few sperm still remain in the subcutaneous connective tissue layer. Externally the flattened empty spermatophore becomes much less conspicuous, and no longer projects appreciably. When ecdysis occurs the ruptured cuticle and adherent spermatophore wall are shed, and the site of penetration of the spermatozoa has a normal or slightly irregular surface.

The passage of the spermatozoa from the vascular spaces of the body to the ovary is probably fairly direct. Spermatozoa are not found in the haemocoel far from the ovary, and their occurrence in connective tissues of the body wall is limited to the vicinity of empty spermatophores. Presumably the spermatozoa are chemically attracted to the ovary. It is believed that they can swim against the blood stream, as they have been

found in channels in the dorsal part of the pericardial network after leaving a dorsally situated spermatophore. There is reason to believe that blood flows through the pericardial network from below upwards, so that these spermatozoa probably were not carried passively to this position.

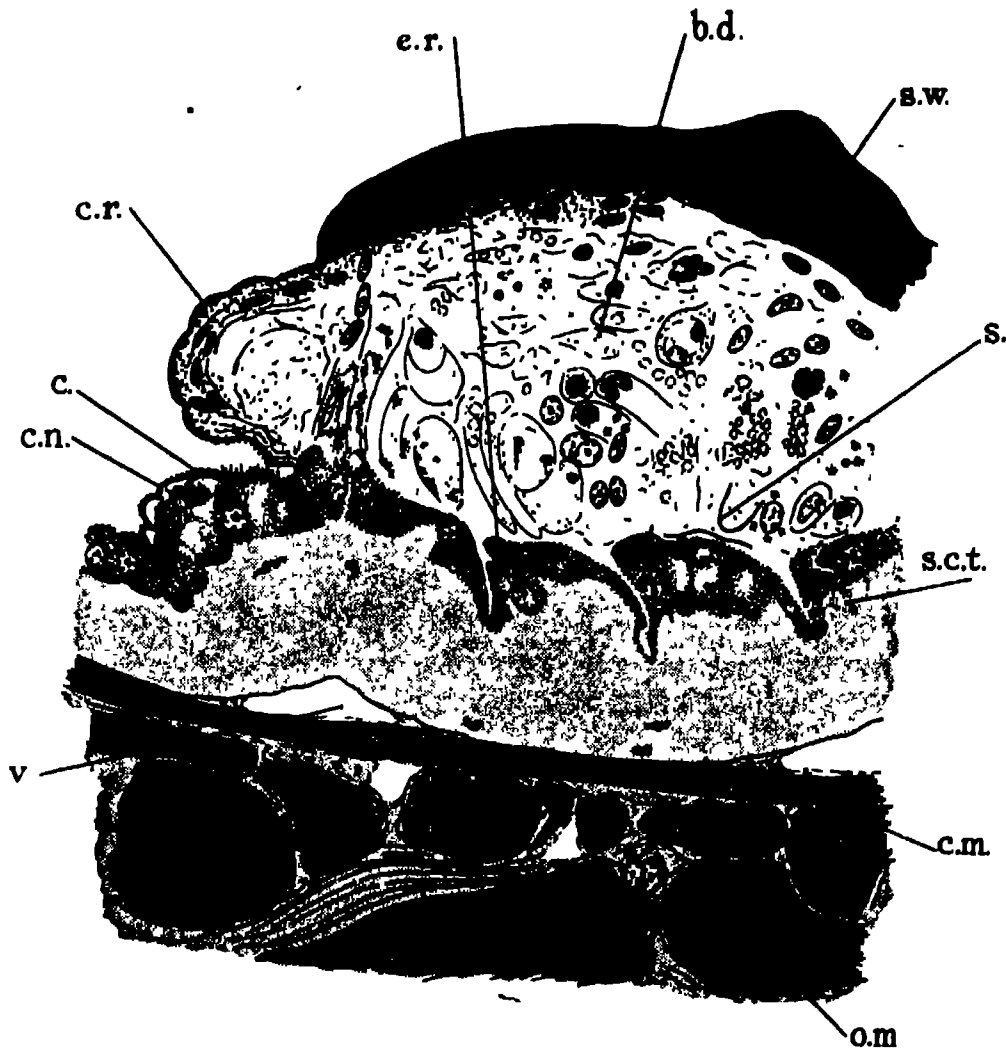


FIG. 3—Vertical section of half a large spermatophore at least ten days after deposition.
For description see text. $\times 392$ approx.

The spermatozoa form a dense felt outside the ovary over the region bearing egg follicles (fig. 15, Plate 51, for structure of ovary see pp. 428 and 431). The follicles may barely project through a tangle of sperm heads $50\text{--}80\mu$ thick. The outer spermatozoa are free, but the inner ones penetrate the much distended cytoplasm of the outer epithelium (fig. 15, the tails of the spermatozoa are not shown). Groups of these spermatozoa become orientated radially and force their way inwards. The middle layer of connective tissue and muscle is bulged inwards and finally broken through, and clumps of sperm find their way into the germinal epithelium. Here they lie in masses in intracellular spaces, and

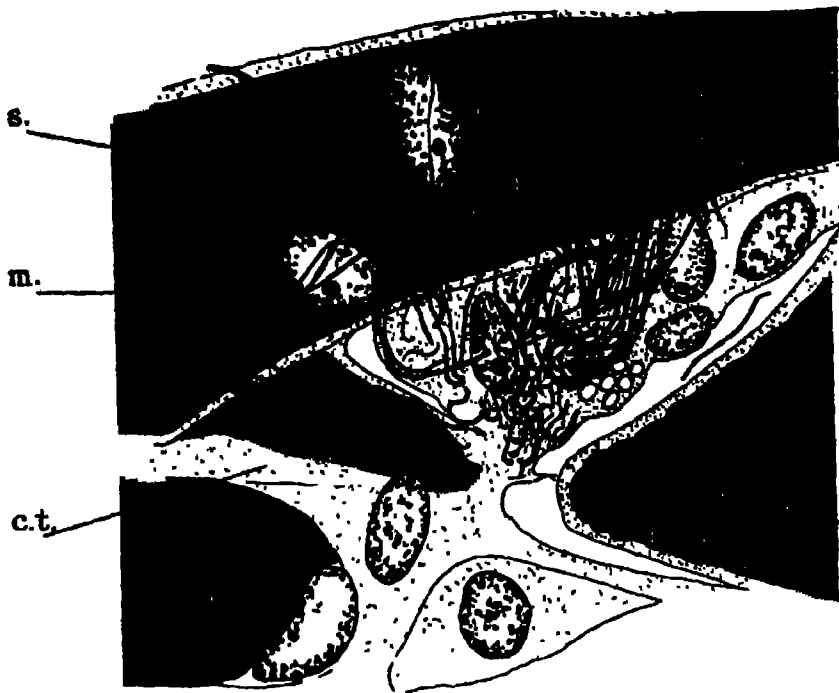


FIG. 4—Section showing a group of spermatozoa (only the heads are drawn) lying between the body wall muscles in the vicinity of an empty spermatophore. $\times 1204$ approx.

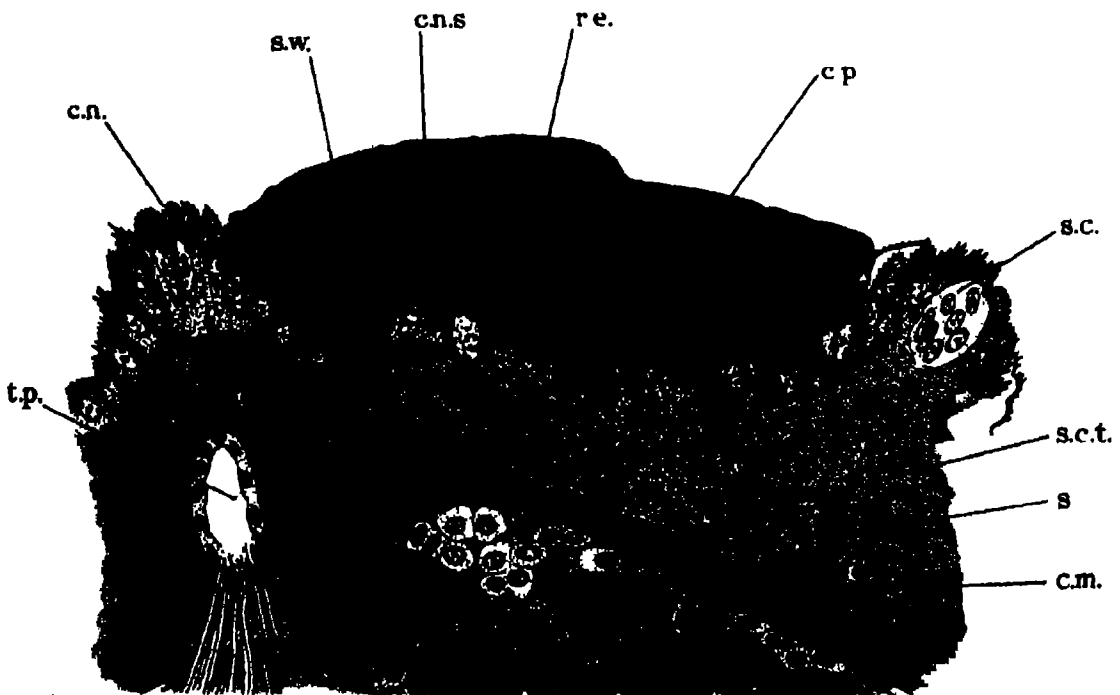


FIG. 5—Vertical section of a spermatophore older than that shown in fig. 3.
For description see text. $\times 350$ approx.

some spread singly through the cytoplasm of these cells. Other clumps of sperm break right through the germinal epithelium and reach the ovarian lumen. An empty channel left by entering spermatozoa is seen in fig. 16, *s.p.p.*, and an entering clump of sperm is shown at *s.p.* The bulk of sperm entering an ovary by this means may be great; figs. 15 and 17 show ovaries on the same scale before and after the immigration of spermatozoa. Only half of the lumen is shown in fig. 17. The disorganization of the germinal epithelium caused by the passage of the spermatozoa may be considerable.

Spermatozoa reach the ovary in this manner during the six months or so in which the male genital organs are functional. The bulk of the spermatozoa may be considerable, and as many as 170 spermatophores may be received by one female during a fortnight.

THE OVARY AND THE EARLY DEVELOPMENT OF THE OVA

The structure of the paired ovarian tubes in the Onychophora has been described by KENNEL (1884), GAFFRON (1885), SHELDON (1889), BOUVIER (1904), etc. The tissue lining each ovary in *Peripatus* forms a homogeneous germinal epithelium throughout the ovary, while in *Peripatopsis*, *Peripatoides*, and other genera the epithelium is thin and sterile except for a longitudinal thickened ridge of germinal epithelium which projects into the lumen of the ovary. The fixation of the ovaries previously described does not appear to have been satisfactory. No cell boundaries could be distinguished by SHELDON and others, and there is general agreement with BOUVIER's statement that "Toutes les cellules épithéliales...de l'épithélium germinatif...semblent identiques et capables de produire un œuf". The earliest recorded ova lie in this germinal epithelium. The later development of the ova in *Peripatus* (where they remain small) takes place entirely within the germinal epithelium, while in the other genera where the ova become larger the later stages are passed in follicles or ovisacs, projecting from the ovary into the haemocoel.

Well-preserved material of *Peripatopsis sedgwicki*, *P. moseleyi*, and *P. balfouri* has been compared with the sections prepared by SEDGWICK and SHELDON, and has considerably extended our knowledge of the structure of the ovary and of the development of ova. The use of Mallory's triple stain was invaluable for ascertaining the extent of the connective tissue in the ovary and for distinguishing between various egg membranes. Cell limits are perfectly definite, and the germinal epithelium is not a spongy syncytium. Nuclei are always present in developing ova and do not entirely disappear at certain stages. These two erroneous conclusions of previous workers were due to the methods employed. The characteristics of the development of ova in the Onychophora listed by SHELDON and BOUVIER, together with the deductions made, need considerable revision.

The paired ovaries of the various species of *Peripatopsis* are very similar in structure. They are tubular, and continuous with their oviducts; they are always united together posteriorly, and occasionally in other places as well.

SEDGWICK (1888) described the embryonic ovary of *P. capensis*. He followed the migration of the embryonic germ cells to the dorsal coelomic pouches of segments 16 to 20, and states that "the cells of the latter form capsules surrounding the germinal nuclei." He figures the ovary as a tube with large germ cells distending the thickened inner germinal ridge.

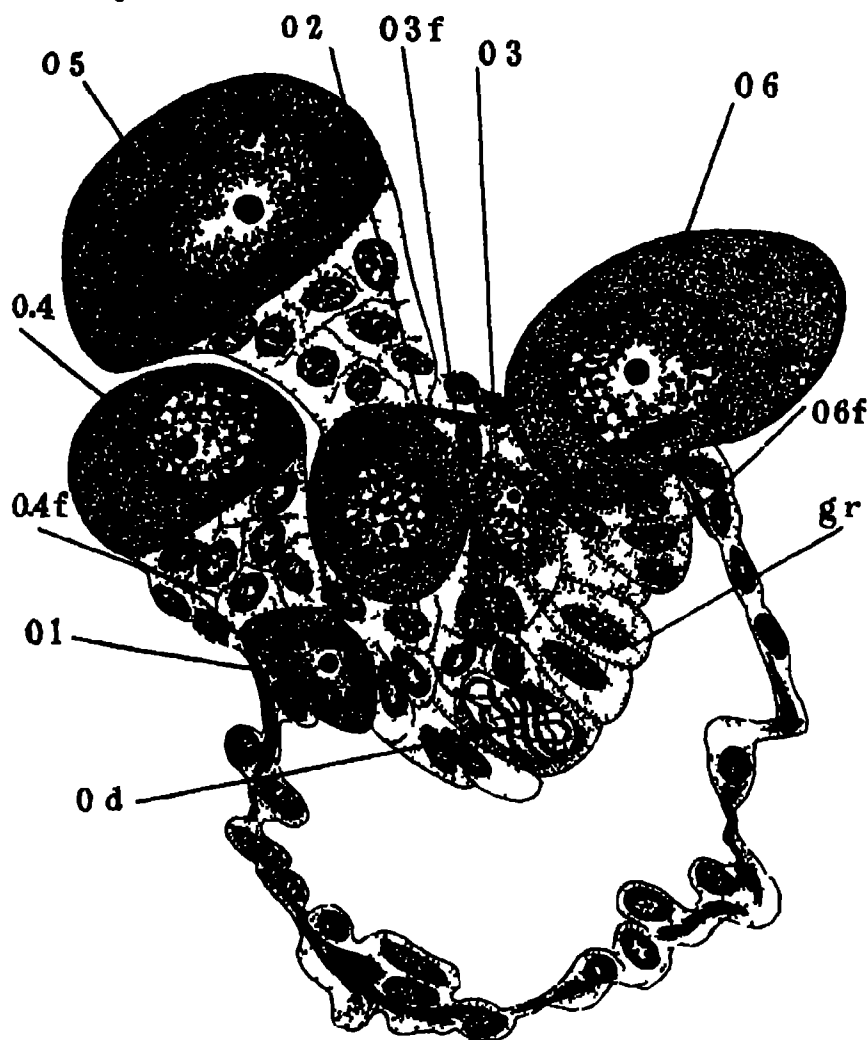


FIG. 6.—Transverse section of one ovarian tube of a recently hatched *P. balfouri*. No ova or oogonia lie in the ovary lumen, both are seen only in the germinal ridge. Two ova are already extended on follicles, but the egg membrane is not yet formed. $\times 980$

The germ cells are at a later stage in the young of a number of species of *Peripatopsis* which have been examined just before and shortly after birth. All these ovaries show the germ cells in the germinal ridge to be quite distinct from the surrounding epithelial cells, just as figured by SEDGWICK. Many of the smaller germ cells, however, are now undergoing fission (fig. 6, *o d*) and others have increased in size. Each of the larger ova

is associated with a group of small follicle cells (fig. 6, *o.6.*, *o.6.f.*, *o.3.*, *o.3.f.*, etc.), and the largest ova project from the ovary into the haemocoel, the follicle cells forming a stalk. It is to be noted that all the ova are situated either in the germinal ridge or projecting externally from it in follicles, and that there are no cells of any kind free in the lumen of the ovary. The ovary is also devoid of projections other than the egg follicles. At the age of two days *Peripatopsis* possesses ova resembling in size and all other respects the stage shown in fig. 18, Plate 51, drawn from an ovary of a full-grown animal.

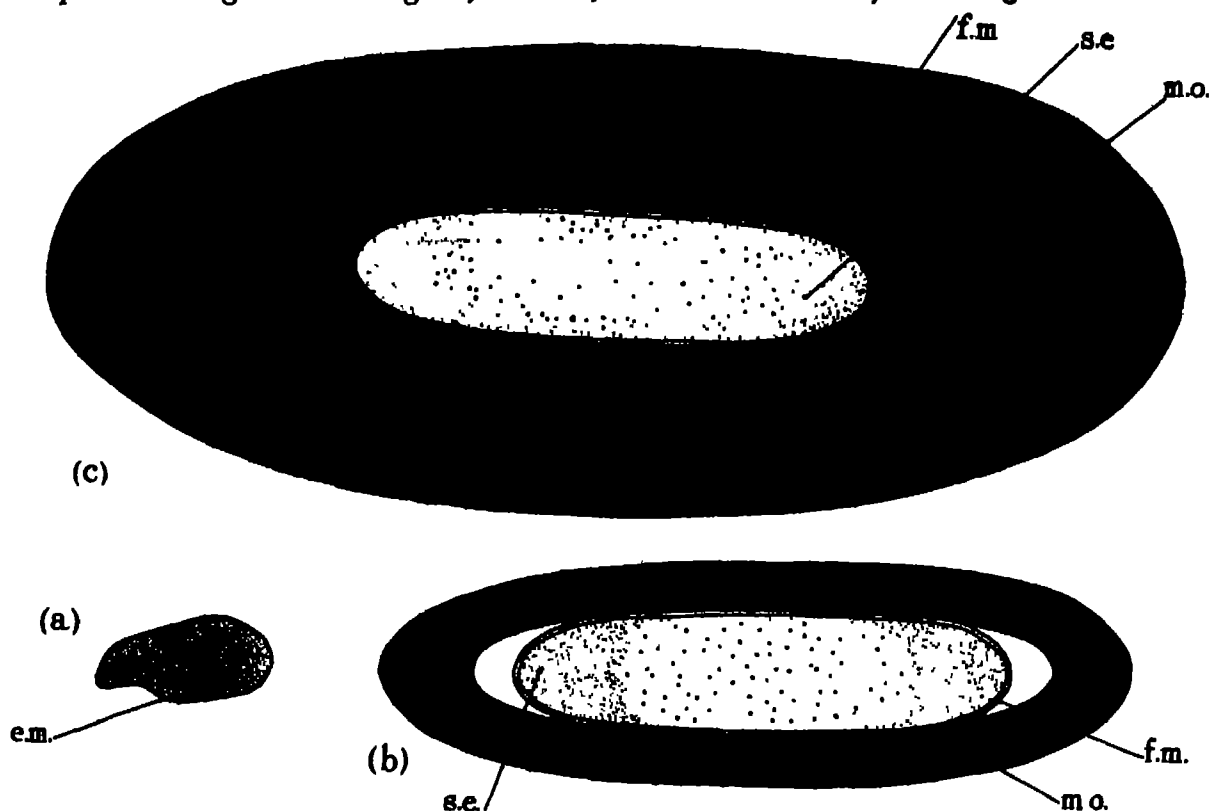


FIG. 7a—Ripe ovum from the lumen of the ovary of *P. sedgwicki*. The ovum has just vacated an egg follicle and the egg membrane is very thin at one end prior to the egg emerging from this membrane for fertilization. $\times 246$ approx. (See also ripe ovum in fig. 19, Plate 51).

FIG. 7b—Diagram of a living 4-6-celled stage from the upper oviduct of *P. sedgwicki*. The embryo has swelled greatly (cp. fig. 7a) and is now enclosed in another membrane (*f.m.*) (possibly a fertilization membrane) and is surrounded by a second thick membrane secreted by the oviduct (*m.o.*). $\times 246$ approx.

FIG. 7c—Diagram of living early segmentation stage of *P. sedgwicki* slightly older than the preceding. The segmenting egg is about the same size but shell secreted by the oviduct is much thicker. $\times 246$ approx.

Up to the age of 6-10 months the ovary maintains the same appearance, except that the ova projecting in follicles may be larger in the older animals. After an animal has copulated towards the end of its first year, the course of the early development of ova changes. This new method continues throughout adult life, and will be described in detail below.

The structure of the ovary of an adult animal differs slightly from that of the young animal which has not copulated. Each adult ovarian tube is formed by (1) an outer irregular epithelium which covers (2) a connective tissue layer bearing muscles running in various directions, and within this lies (3) the inner epithelium. Tracheae are absent from the ovary although the oviduct is abundantly supplied. The sterile parts of the inner epithelium are usually not more than one cell thick, but may be irregular (figs. 15, 17, Plate 51). The thickened ridge of germinal epithelium, which is ventral in the embryo, lies in the adult on the side of the ovary remote from its fellow, and varies greatly in its extent and thickness. Tall columnar cells form pad-like projections which may be many cells thick, and tongues of connective tissue may extend inwards from the middle layer to support these thickenings.

The sterile region of the ovary wall may show short diverticula projecting outwards. These lobes have little or no muscle, they are thin-walled and often filled with sperm (figs. 15, 17, *s.o.d.*). Outside the germinal ridge the ovary wall may bear many and varied projections in animals which have previously given birth to young; many of these are indirectly due to the formation of the projecting follicles which are ultimately vacated by the ripe ova, and will be described below (p. 435). The lumen of the ovary may be large or small, varying with the abundance of contained spermatozoa.

At the age of a little less than one year, when the lumen of the ovary has been filled with sperm, strands of cells from the germinal ridge migrate into and around the sperm mass. These cells become detached from the ovarian wall, take on a round and undifferentiated form, and lie freely in the lumen among the sperm. Later they give rise to ova. The origin of these free oogonia has been observed clearly in only one individual aged about 11 months. Free oogonia together with all stages of development of mature ova from these cells have been followed in many full and partly grown animals of two or more years in age. Whether the oogonia pass into the lumen of the ovary repeatedly throughout life, or only during the first year, is not known. Some germinal epithelial cells become detached at the times when spermatozoa penetrate through the ovary wall, a process causing some disruption of the tissues, but their fate is unknown. In some ovaries free cells are found which resemble blood leucocytes in size and staining reactions, and which may show a lobed nucleus as do many of the leucocytes (see MANTON and HEATLEY 1937, fig. 23). These leucocyte-like cells have never been seen to contain sperm heads.

The youngest oogonia observed in all adult animals examined were lying freely in the lumen of the ovary. None were found in the germinal epithelium, their position in late embryos and during the first few months after birth. Free oogonia occur either singly or in masses, but are always separate from the wall of the ovary. The oogonia may be abundant in parts of an ovary, several dozen touching one another being apparent in one transverse section, and in other parts of the same ovary they may be few and scattered. The oogonia are surrounded by sperm as in fig. 17. The smallest oogonia are almost indistinguishable from free epithelial cells, and have been seen most

abundantly in ovaries of *P. moseleyi* in February and of *P. sedgwicki* in March and May. This extensive range of months is probably associated with the breeding season of these two species being less restricted than it is in *P. balfouri* and *P. capensis*, the young in the two former species being born from June to September and July to December respectively.

The oogonia are spherical, unless distorted by pressure, and are about $18\text{--}20\mu$ in diameter. The cytoplasm is evenly granular, and the nucleus, with characteristic nucleolus, is about $8\text{--}10\mu$ across. The nuclei of the oogonia are usually quite distinct from those of the germinal epithelial cells where they are various in size, and the nucleolus, if present, is smaller and less conspicuous (fig. 13, Plate 50, *g.r.n.*). The cytoplasm of the oogonia becomes packed with sperm heads. The length of the head is about $75\text{--}100\mu$ (fig. 14), and thus each is folded or curled round the nucleus of the oogonium. It was impossible to count the number of sperm heads within an oogonium, but there must be several. Those shown in figs. 8–10, lie in one plane only, and many others were present on either side, but they have been omitted from the figures for the sake of clarity. Some of the sperm heads appear partly to lie outside the oogonia. Probably these are actual cases of penetration of the oogonium by a spermatozoon, but the abundance of sperms outside the oogonia as well as inside, and the great length of the sperm head which may extend through ten sections, makes a critical statement impossible. The oogonia divide to give descendants like themselves. During cell division the sperm heads lie in the peripheral cytoplasm outside the mitotic figure, and do not appear to hinder this process. Two daughter nuclei are seen in fig. 9. This stage is followed by division of the cytoplasm, and the sperm heads are shared between the daughter cells.

The young ova so formed in the lumen of the ovary increase in size to an average diameter of 32μ in *P. sedgwicki*, but sizes up to $54 \times 40 \times 40\mu$ have been found. The nucleus increases to about 20μ or more, but its appearance is otherwise unaltered. The ova are not further invaded by sperms although they are still surrounded by them, and the contained sperm heads gradually become fewer and finally are entirely absorbed. Stages of this process are shown in figs. 10–12. Some oogonia and early ova show spherical regions in the cytoplasm which stain light or dark blue with Mallory's triple stain (figs. 9, 10). These patches disappear as soon as the sperm heads are all absorbed. Probably they represent some of the material of the sperm head in the process of absorption. In the bed-bug the absorption of sperm heads leaves stainable zones within the absorbing cell (ABRAHAM 1934).

The ova now become attached singly to the thickened germinal ridge. They adhere to the exposed ends of the epithelial cells (fig. 12), and in their vicinity a number of small "follicle" cells (*f.c.*) appear with darkly staining nuclei and cytoplasm less dense than elsewhere. These cells spread round and over each ovum and finally enclose it (fig. 13). The ovum at this stage is about $32\text{--}45\mu$ in diameter in *P. sedgwicki*. The follicle so formed usually separates the ovum from the spermatozoa in the lumen of the ovary,

as in fig. 13, but in some cases a thin but dense investment of spermatozoa lies round the ovum inside its follicle, although these do not penetrate the egg.

The ovum with its follicle cells now sinks farther into the thick germinal epithelium, and comes to lie against the connective tissue layer which separates the inner ovarian epithelium from the outer epithelium. The follicle cells are now situated mostly on the side of the ovum facing the ovarian lumen (fig. 18, Plate 51), the reverse of their original position. The ovum is pushed through the layer of connective tissue and muscle, followed by a plug of follicle cells, and reaches the outer epithelium. This stage represents the earliest one found by previous workers (see SHELDON 1889, Pl. 29, fig. 3), who could not distinguish cell limits in their material and thus could not follow the details of ovary structure and the movements of ova. The size of the ovum increases during this migration to an average of 38μ diameter in *P. sedgwicki*, but smaller and larger specimens have been found measuring $30 \times 30 \times 25\mu$ and $54 \times 34 \times 48\mu$ respectively.

The follicle cells now swell and increase in numbers, and force the ovum away from the general ovarian surface. The beginning of this process is seen in fig. 18 where the ovum is about 38μ in diameter. A thin layer of connective tissue is left covering the ovum as a result of its passage through the ovarian wall, and this connective tissue forms a permanent investment to the projecting follicle, and remains continuous with the main connective tissue layer. Some of the follicle cells shown in fig. 18 still lie in the inner epithelium. These gradually become external and ultimately form a stalk about 120μ long in *P. sedgwicki*. This follicular stalk is only one cell thick ($20\text{--}30\mu$) and is flat and wide ($70\text{--}80\mu$) (see fig. 17).

When the follicle stalk is fully extended a change takes place in both follicle and ovum. The terminal follicle cells lying against the ovum cease to resemble the rest, and form a regular darkly staining layer, and a space appears between this layer and the opposing wall of the ovum. The latter usually becomes flattened on this side, and acquires a thick membrane staining blue with Mallory and brownish with iron haematoxylin. This membrane has been recorded by SHELDON as the "egg-shell", and others have assumed that fertilization is effected through it, and that it persists into embryonic life. The egg with its membrane then increases in size to a maximum of $70\text{--}80\mu$ in diameter in *P. sedgwicki*. There is no evidence to support BOUVIER's suggestion that the ovarian egg grows to 600μ in this species. In *P. moseleyi* the follicular egg reaches 150μ in diameter.

The egg remains in this position until 1 or 2 months before the birth of the uterine embryos. The follicle and connective tissue sheath may shrink away from the egg membrane all round, and the ovum then becomes spherical. The nucleus may become greatly distended so that it is not readily distinguishable from the cytoplasm, but it never disappears.

Two specimens of *P. sedgwicki*, "A" killed in January and "B" killed in March, and one specimen of *P. moseleyi* killed in February showed advanced embryos in the lower oviduct, while the upper oviduct contained the earliest embryonic stages recorded for

these species. In *P. sedgwicki* "A" about a dozen 4-6-celled stages lay in the upper oviduct, and the ovary lumen contained a few unfertilized eggs which had not yet passed into the oviduct. In *P. sedgwicki* "B" the upper oviduct contained early segmentation stages, and the ovary lumen was devoid of mature ova. In both animals all the projecting ovarian follicles were empty, and therefore ovulation must have been completed. In the *P. moseleyi* only two unsegmented eggs lay in the upper oviduct, and the ovarian follicles showed a few ova; thus in this animal ovulation had only just begun.

The further history of the ova after their growth in the follicles appears to be as follows: over the whole ovary the cells of the follicle stalks alter their arrangement and form hollow tubes instead of flat plates. Each ovum covered by its thick membrane passes through the follicle stalk into the ovarian lumen, going through the gap in the connective tissue through which it previously came. The germinal epithelium may be disorganized by the passage of the large ovum; and the follicle remains as an empty diverticulum of the ovary (fig. 19). The ova in the ovarian lumen in *P. sedgwicki*, specimen A, measure $65-80\mu$ in diameter and most are slightly elongated. They are surrounded by spermatozoa and by cells, partially or completely detached from the germinal epithelium. The egg membrane is entire and lacks a micropyle, and the nucleus of each ovum is so far unaltered (fig. 19).

The ovum then swells slightly, and the egg membrane becomes thin and stretched at one end (fig. 7a). It is believed that the ovum then emerges from its membrane. Empty membranes have been found in the ovarian lumen corresponding roughly with the number of young segmentation stages in the upper oviduct, but neither could be counted exactly. It is probable that the naked egg is then fertilized by a spermatozoon, and that it rapidly passes into the oviduct.

The next stage seen was also found in the females mentioned above. The 4-celled egg in *P. sedgwicki* had swollen to $260 \times 80 \times 80\mu$ (fig. 7b), and the unsegmented egg in *P. moseleyi* measured 520μ . This sudden swelling was noted by SHELDON for *P. capensis*, but it was ignored by BOUVIER whose conclusions in consequence are erroneous.

The fertilized and segmenting eggs in the upper oviduct are covered by two membranes. A thin inner membrane stains red with Mallory, in contrast to the ovarian egg membrane which stains blue. An outer membrane is progressively secreted by the oviduct wall and rapidly attains the enormous thickness of $140-240\mu$ in *P. sedgwicki* and stains blue with Mallory (fig. 7b and c). This outer membrane stretches as the segmenting egg swells to a length of 2.5-3 mm., and remains intact until shortly before the birth of the young. The substance of the egg within the inner membrane is at first very diffuse owing to the rapid swelling, and masses of protoplasm containing chromosome or nuclear spheres lie scattered through the fluid contents. SEDGWICK had the good fortune to kill an animal at a stage in which the upper oviduct ova were showing maturation and fertilization phenomena (SHELDON 1889). His stages are intermediate between the ovarian and uterine ova from specimen A of *P. sedgwicki* described above. SHELDON's

preparations have been re-examined and they show that the maturing egg with its male and female pronucleus is surrounded by a membrane stained with haematoxylin which she did not figure. It is probable that this single membrane covering the egg after fertilization is the inner membrane of the enlarged segmenting egg shown here in fig. 7c which also stains with haematoxylin (a blue-black colour). It is certainly not the membrane covering the ovarian egg, as this is left in the ovary and has different staining properties, appearing blue with Mallory while the inner membrane of the uterine egg stains red. The latter may be a fertilization membrane. It disappears later when the segmenting egg has increased to a length of 2.5 mm.

The emptying of all the ovarian follicles at ovulation leaves the ovary covered with empty and shrunken diverticula. These may become more or less solid projections which remain on the ovary (fig. 15, Plate 51), or they may be invaded by few or many spermatozoa from the lumen of the ovary. The proximal parts may be distended by sperm and then the distal regions become solid or shrink, or the distal parts may become distended, the wall remaining thin; the whole then externally resembles an egg-containing follicle, and may even exceed the latter in size. Most of the spermatozoa remain in the lumen of these lobes, but a few penetrate the follicle cells.

It is not known whether these effete follicles are ever completely absorbed. Some of them may be, but an old animal shows many more sterile than fertile follicles attached to its ovaries. The passage of the ova from the follicles to the ovarian lumen is followed by fresh ova becoming attached to the germinal epithelium, and the subsequent formation of new follicles in the manner described.

The number of follicular ova on one ovary varies with the condition of the animal, a feeble starved specimen sometimes showing fewer ova than normal. In all well-fed individuals the number of follicular ova exceeds the number of segmenting eggs in the upper oviducts. The latter correspond closely to the number of embryos in the lower oviducts. If all the follicular ova pass into the ovary at ovulation, as is suggested by the specimens examined, some of these ova must fail to give rise to segmenting eggs. The fate of such surplus ova is unknown. Absorption of germ cells occurs in other animals (e.g. *Hemimysis*, see MANTON 1928, but here it takes place at an earlier stage).

In all adult animals examined the oogonia and early ova contain sperm heads in their cytoplasm at stages younger than that of fig. 12, Plate 50. Whether in adult animals the ova can develop and give rise to normal eggs in the absence of spermatozoa, as they do in newborn and very young animals, or whether their growth is entirely dependent upon the sperm they normally ingest is not definitely known. Examination of one animal indicates that the latter may be the case. A 3 or 4 year-old *P. sedgwicki*,* which had previously produced normal young, and which contained late embryos in the oviduct, refused to feed and was dying of starvation. On killing, the ovary was found to be devoid of sperm, although copulation had been frequent. Free oogonia and young ova, which normally contain sperm heads, were absent, and only a few ova, not more

* *Peripatopsis* normally lives for at least six years and reproduces up to at least the sixth year.

than seven, lay in the germinal ridge.* A reduced number of fertile ova had just passed into the upper oviduct. In this case it appears that (1) the animal had absorbed ovarian spermatozoa when food was not eaten, and (2) that the development of young ova ready to penetrate the germinal ridge had not taken place normally in the absence of abundant ovarian spermatozoa. Thus a certain number of spermatozoa may be necessary for absorption by the ova and oogonia of adult animals.

The sequence of events described above for the development of ova is quite definite. A complete series of stages has been examined for *P. sedgwicki*; the free ova and all the follicular stages have been seen in *P. moseleyi*; naked ova becoming attached to the germinal ridge (as in fig. 11), and all subsequent follicular stages have been seen in *P. capensis*; and follicular stages alone have been examined in *P. balfouri*.

The duration of the various stages of ovum development is less certain. The follicular growth stages of *P. capensis* described by SHELDON (1889) as occurring progressively during a period of about 9 months from July to the following April, correspond with those of *P. sedgwicki* starting at a stage a little younger than that shown in fig. 18. It is probable, for the reasons given below, that the duration of ovum development is not rigidly fixed or always so long, and that in *P. sedgwicki*, *P. moseleyi*, and *P. balfouri*, and probably in other species as well, the development of ova ready for fertilization may occur quite rapidly instead of taking more than 9 months.

1—Some individuals of *P. sedgwicki* and *P. moseleyi* killed from January till July show all stages between early oogonia and stalked follicles bearing full-size ova on the same ovary. Thus these stages here do not appear to be reached progressively at certain months in the year in any one animal. Only in ovaries from animals which had recently given birth to young were a limited number of stages found. Here all the ova in the germinal ridge and on follicles were small and young, and a relatively small number of follicular ova had so far been formed.

2—The ovary of a *P. balfouri*, killed in June and carrying segmentation stages in the oviduct, showed all stages of follicular ova, but no free ova or oogonia in the lumen. If all the ova pass from the follicles at ovulation in *P. balfouri* as they do in the specimens of *P. sedgwicki* here examined, then the fully formed follicular ova in June must have developed since the last ovulation, a period of about 2–3 months, and unless they are absorbed must remain in this state until the next ovulation, which will occur in about 9–10 months' time.

3—Projecting follicular ova are well developed in animals a few days after birth. Such ova are absent in advanced embryos, and thus must develop in a few weeks or days.

It is thus probable that the development of ova from oogonia to fully formed follicular stages may take place both rapidly and at various times in the year, and that the ova surrounded by a membrane on the projecting follicles may remain dormant in this position for several months before passing back into the ovary for fertilization.

* The ova in or attached to the germinal ridge are normally too numerous to count.

THE ABSORPTION OF SPERMATOZOA IN THE OVARY

It has been noted above (p. 428) that the bulk of spermatozoa reaching the ovary during the year may be great. It is unlikely that the large mass of spermatozoa which may be found in the ovaries of *P. sedgwicki* or *P. moseleyi* is ever completely absorbed at one period during the year. No ovary was devoid of spermatozoa in animals aged 6 months and upwards. SEDGWICK (1885) noted that spermatozoa occur "in smaller numbers directly after the eggs have passed into the oviduct than at any other time". SEDGWICK's preparations have been re-examined, and they show a much smaller accumulation of spermatozoa than is found at times in the species here described (see fig. 17 and compare with SHELDON 1889, Pl. 30, fig. 22).

It is evident that a large amount of spermatozoa disappear from the ovarian lumen, but the manner of utilization of so much sperm is not fully understood. An insignificant number fertilize the 20-60 mature eggs, and a greater number penetrate the developing oogonia and are absorbed by them, but the extent of such absorption cannot be estimated. It is probable that the majority of spermatozoa are absorbed by the ovary in some other manner. The cells of the inner ovarian epithelium frequently contain single sperms within their cytoplasm or clumps of sperm lying within large vacuoles, but there is no indication as to how far these sperm are in process of penetration to the lumen or being absorbed by the epithelium. It has already been noted that during starvation absorption may be abnormally great, so that although copulation is frequent the ovary may be devoid of sperm.

DISCUSSION

Fertilization in many groups of invertebrates is obtained by the passage of spermatozoa through the body wall of another individual. Spermatozoa may be injected anywhere into the body by a muscular penis, as in *Dinophilus*, Rotifers and many Turbellaria. In the bed-bug *Cimex* the spermatozoa pass through the body wall by their own activity, making their way through the cells of the organ of Berlese to reach the haemocoel. In other invertebrates spermatophores may be employed as a means to internal fertilization. In leeches such as *Clepsine* and in *Peripatopsis* closed spermatophores are placed anywhere on the body, and their contained spermatozoa pass through the body wall. In many Ichthyobdellid leeches a specialized "tissu vecteur" is provided in the clitellar region for the passage inwards of the sperm (BRUMPT 1900).

Peripatopsis thus resembles *Cimex* and *Clepsine* in that the spermatozoa from the spermatophores pass into the body by their own activity, but the details of this process in *Peripatopsis* are unlike those in any other animal described. In *Cimex* a cytoplasmic surface is provided by the organ of Berlese, while in *Clepsine* and *Peripatopsis* the spermatozoa have to traverse the cuticle and body wall. In *Clepsine* (WHITMAN 1891) and *Placobdella* (MEYERS 1935) this is a rapid process, and starts a few minutes after deposi-

tion of the spermatophore. Spermatozoa stream through the skin for about one hour, and the waterproof spermatophore wall contracts and appears to force the sperm into the body. A small initial perforation of the cuticle may or may not be made by the hard spermatophore on deposition (MEYERS 1935). The rapid perforation of the body wall is caused by a secretion of the epididymis which causes histolysis of the tissues in the neighbourhood of the spermatophore attachment. In *Peripatopsis*, although the integument is very thin, the passage inwards of the sperm is delayed for 7–10 days until leucocytes have caused sufficient breakdown of the ectoderm and integument to allow the sperm to swim freely into the haemocoel. No further breakdown of the tissues takes place and no cytolytic agent is secreted by the male. Thus the penetration in *Peripatopsis* is very different from that in the leech, and in neither *Peripatopsis* nor *Cimex* do the sperm themselves penetrate through tough membranes.

In *Cimex*, *Clepsine* and *Peripatopsis* the sperms reach the ovary by their own activity, and after traversing the body spaces they penetrate through the ovary wall. In *Peripatopsis* they pass through this wall in clumps rather than singly, just as described by CRAGG (1920) for the organ of Berlese of *Cimex*. In *Cimex* the spermatozoa from the haemocoel penetrate into the "resorptions Organe" (previously called "spermathecae", see ABRAHAM 1934), and some of them pass on up the ovariole wall intracellularly and reach the developing ova.

Fertilization of the ova in *Peripatopsis* undoubtedly takes place after each egg has vacated both follicle and egg membrane and is about to enter the oviduct. It is an event completely dissociated from the early absorption of sperm by the cytoplasm of the oogonia and ova. In these early stages the nucleus remains unchanged throughout the period of sperm absorption which is completed weeks or months before fertilization. In *Cimex* ABRAHAM (1934) suggests that polyspermy occurs in the ovariole, and that of the many sperms entering the egg only one effects fertilization, the rest being absorbed. Thus here the egg receives spermatozoa but once. Details of these events in *Cimex* have not been observed beyond the disappearance of spermatozoa near the developing ova.

It was first suggested by CRAGG (1923) that spermatozoa provide nourishment for the developing ova of *Cimex*. When the supply of spermatozoa is becoming exhausted in a well-fed bug the animal ceases to lay normal eggs and produces small abnormal and sterile ones instead. Unless spermatozoa are used to nourish the ova it is difficult to account for the formation of ill-developed eggs in their absence. The only direct observation supporting this suggestion is the continual migration of spermatozoa up the ovariole wall and their disappearance in the vicinity of the developing ova. In the adult specimens of *Peripatopsis* here described, the early development of the ova is associated with the absorption of sperm by the cytoplasm of the ova, and a lack of spermatozoa in the ovary is associated with an absence of young ova. It is thus possible that in *Peripatopsis* as well as in *Cimex* the normal development of ova is dependent upon sperm absorption. In newborn *Peripatopsis* young ova develop directly in the germinal ridge without sperm absorption. This may be regarded as the normal or

primitive method of egg development which probably preceded the present adult condition where sperms are absorbed.

Copulation in *Cimex* and *Peripatopsis* is frequent, and a great volume of spermatozoa is absorbed and disposed of by the female genital organs of both these animals. It has been shown by ABRAHAM (1934) that most of the spermatozoa in the female *Cimex* are normally absorbed by the "resorptions Organe". Here the sperms become motionless within two days and subsequently disappear. Absorption also takes place in the haemocoel by means of leucocytes. In *Peripatopsis* no special organs are developed for sperm absorption which must take place in the ovary, and in the haemocoel no leucocytes have been seen to contain sperms. The physiological significance of this utilization of spermatozoa is unknown. Spermatozoa are not retained intact by starving females of *Peripatopsis* or *Cimex* (CRAGG 1923) which are copulating freely. Here the sperm may be used for supplying nourishment or other substances to the female. In mammals and some other animals a small number of spermatozoa are absorbed by cells of the female genital tract, but this absorption, unlike that of *Peripatopsis* and *Cimex*, has little, if any, physiological significance.

SUMMARY

1. The passage of spermatozoa to the ovarian lumen from the spermatophores attached to the body of the female is described.

2. Following the deposition of a spermatophore, leucocytes invade the subcutaneous region and break through the ectoderm. The cuticle of the body and the lower wall of the spermatophore are ruptured.

3. Spermatozoa swim from the spermatophore through the perforated cuticle and ectoderm and reach the vascular spaces. They pass through the haemocoel by their own activity and reach the ovary.

4. Clumps of spermatozoa force their way through the ovarian wall to reach the ovarian lumen.

5. The spermatophore wall remains attached to the cuticle, so closing the wound caused by the entry of the spermatozoa. The ectoderm regenerates and forms a new cuticle which is exposed at the next ecdysis.

6. The earliest oogonia in adult animals lie freely in the ovarian lumen among the spermatozoa. Their cytoplasm is invaded by sperm heads. They divide to form ova which absorb the sperm heads and grow.

7. Each ovum sinks into the germinal ridge epithelium. The formation of the egg follicle and ovarian egg membrane are described.

8. The ripe ovum passes through the follicle stalk back to the ovarian lumen. Here it emerges from the egg membrane. Fertilization takes place and the egg passes down the oviduct. The fertilized egg swells rapidly and a membrane is formed round it, which is probably the fertilization membrane. The oviduct secretes a second very thick membrane outside the first; only this second membrane persists during embryonic life.

9. The spermatozoa in the ovary (1) fertilize the eggs, (2) provide the early ova with nutriment necessary for their growth, and (3) may supply the animal with nourishment or other special substances.

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KEY TO LETTERING

- | | | | |
|---------------|--|-------------------------|--|
| <i>b.</i> | blister below spermatophores filled with leucocytes. | <i>m.o.</i> | egg membrane secreted by oviduct. |
| <i>b.d.</i> | blister below spermatophore filled with degenerating leucocytes. | <i>o.</i> | ovum. |
| <i>c.</i> | cuticle of body surface. | <i>o.d.</i> | oogonium preparing for division. |
| <i>c.m.</i> | circular muscle. | <i>o.f.</i> | ovum in projecting follicle and enclosed in egg membrane. |
| <i>c.n.</i> | new cuticle of body forming beneath the old one. | <i>o.m.</i> | oblique muscle. |
| <i>c.n.s.</i> | new cuticle formed by regenerated ectoderm below the spermatophore. | <i>o.p.</i> | outer epithelium of ovary wall. |
| <i>c.p.</i> | cuticle below spermatophore showing perforations. | <i>o.1., o.2., o.3.</i> | young ova in germinal ridge. |
| <i>c.r.</i> | cuticle raised up from ectoderm below spermatophore. | <i>o.4., o.5.</i> | older ova on projecting follicles, egg membrane not yet formed. |
| <i>c.s.</i> | connective tissue layer pushed inwards by invading spermatozoa. | <i>o.3.f.</i> | follicular stalk cells of <i>o.3.</i> |
| <i>c.t.</i> | connective tissue. | <i>o.4.f.</i> | follicular stalk of <i>o.4.</i> |
| <i>c.t.m.</i> | connective tissue-muscle layer of ovary wall. | <i>o.6.</i> | ovum starting to project from ovary. |
| <i>d.</i> | diverticulum of ovary wall. | <i>o.6.f.</i> | cells which will form follicular stalk of <i>o.6.</i> |
| <i>d.g.r.</i> | germinal ridge cells displaced by passage of ripe ovum. | <i>p.</i> | passage through ovary wall left by ripe ovum passing from follicle to ovary lumen. |
| <i>e.</i> | ectoderm. | <i>r.e.</i> | regenerated ectoderm below spermatophore. |
| <i>e.f.</i> | empty follicle vacated by ripe ovum. | <i>r.o.</i> | ripe ovum free in ovary lumen but still enclosed in ovarian egg membrane. |
| <i>e.m.</i> | membrane formed round ovum in follicle. | <i>s.</i> | sperm heads. |
| <i>e.r.</i> | ruptured ectoderm. | <i>s.c.</i> | sense capsule. |
| <i>f.</i> | stalk of egg follicle. | <i>s.c.t.</i> | subcutaneous connective tissue layer. |
| <i>f.c.</i> | follicle cells which will pass out behind the ovum to form the follicle stalk. | <i>s.e.</i> | segmenting egg. |
| <i>f.m.</i> | membrane formed round egg after fertilization. | <i>s.g.r.</i> | sperms in cells of germinal ridge. |
| <i>f.o.</i> | free young ova or oogonia in the ovary lumen. | <i>s.l.</i> | sterile projecting lobe from ovary. |
| <i>g.r.</i> | germinal ridge. | <i>s.o.</i> | free sperms in ovary lumen. |
| <i>g.r.n.</i> | nucleus of germinal ridge epithelial cell. | <i>s.o.d.</i> | free sperms in diverticulum of ovary lumen. |
| <i>i.p.</i> | inner epithelium of ovary wall. | <i>s.o.p.</i> | sperms invading outer epithelium of ovary. |
| <i>l.</i> | leucocyte. | <i>s.p.</i> | plug of sperms penetrating through ovary wall which is not yet fully perforated. |
| <i>l.o.</i> | lumen of ovary. | <i>s.p.p.</i> | open passage left by a plug of sperms which has passed into ovary lumen. |
| <i>l.p.</i> | leucocyte containing pigment granules from ingested ectodermal cells. | <i>s.w.</i> | spermatophore wall. |
| <i>m.</i> | muscle. | <i>t.p.</i> | tracheal pit with attached tracheae. |
| | | <i>v.</i> | vascular space in body wall. |

DESCRIPTION OF PLATES

PLATE 50

Sections showing oogonia and young ova of *Peripatopsis sedgwicki*. $\times 1517$ approx.

FIG. 8—Two young ova or oogonia containing sperm heads in the cytoplasm from the lumen of the ovary in May. These cells are surrounded by free sperms and other ova like themselves.

FIG. 9—Oogonium from the lumen of the same ovary in process of division. Nuclear fission is complete and will be followed by that of the cytoplasm. Sperm heads lie in the peripheral cytoplasm, and do not hinder the division. The darkly staining spheres (blue with Mallory) probably represent material from the sperm heads in process of absorption.

FIG. 10—Older ovum from the lumen of the same ovary. Three other ova lie in contact with it, and it is also surrounded by free sperms. The ovum is larger, but contains fewer sperm heads, and the darkly staining zones in the cytoplasm probably represent absorption of sperms. From this stage onwards no further sperms pass into the cytoplasm of the growing ovum, although sperms are in free contact with it.

FIG. 11—Older ovum from lumen of ovary in March. Nearly all the sperm heads have been absorbed.

FIG. 12—Ovum of about the same size as the last which has absorbed all sperms and has become attached to the inner epithelium of the germinal ridge. Many sperms lie in the ovary lumen.

FIG. 13—Ovum at a slightly later stage from an ovary in March. The ovum has sunk into the germinal ridge and become overgrown by follicle cells with small nuclei. The edge of an intracellular space in the germinal ridge which is filled by sperm is cut in this section. (s.g.r.)

FIG. 14—Sperm head of average size drawn to the same scale as the ova here figured.

14



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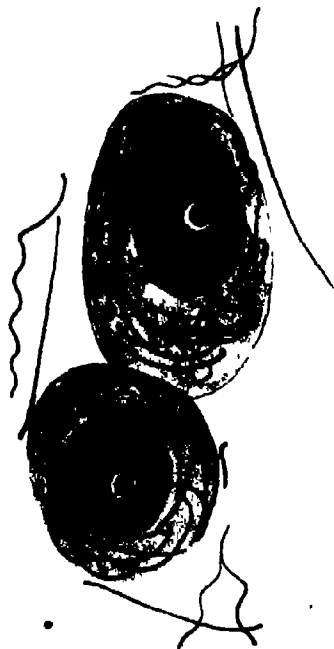
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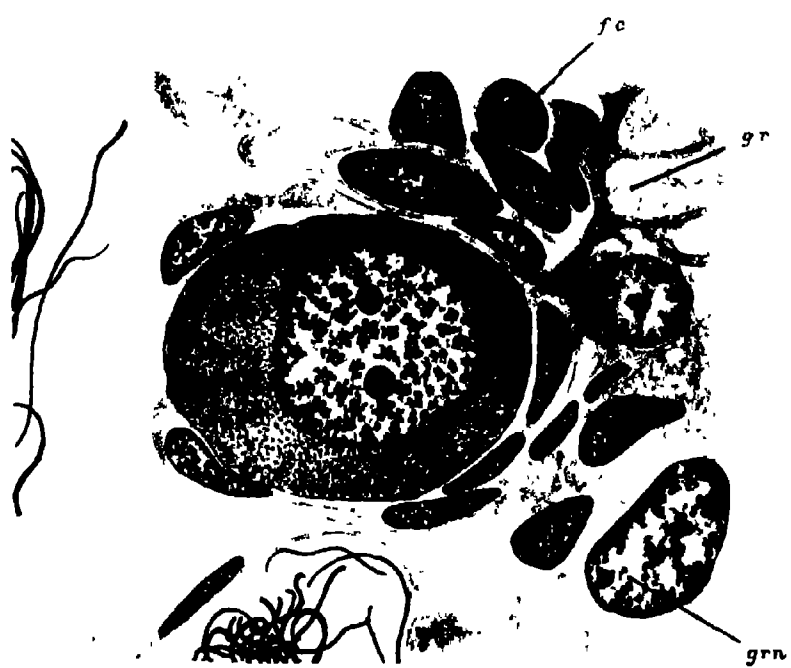
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8



13

PLATE 51

Sections of ovaries of *P. sedgwicki* showing the penetration of sperms into the ovarian lumen from the haemocoel, and the later development of the ova after they have sunk into the germinal ridge.

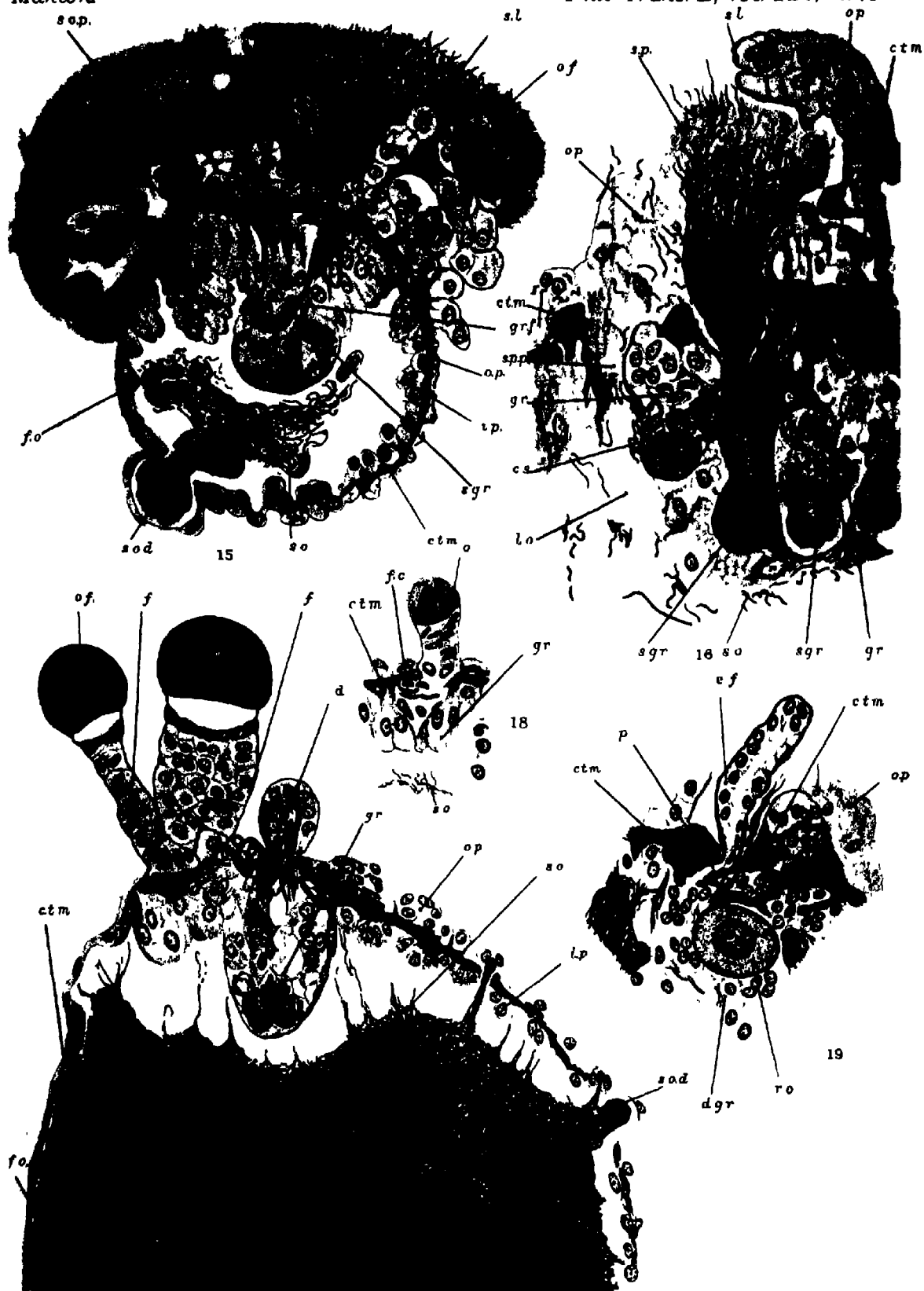
FIG. 15—Transverse section of ovary in July. A few very young ova and sperms lie in the lumen. The ovary is moderately contracted owing to the scarcity of solid contents; the germinal ridge projects into the lumen on one side, and externally project many sterile lobes (*s.l.*) and fertile follicles (*f.*). The cytoplasm of the outer epithelium of the ovary wall is swollen on the follicle side of the ovary, and is distended with a mass of sperm heads which will later pass into the ovarian lumen. $\times 290$ approx.

FIG. 16—Transverse section of part of the ovary wall in March showing the penetration of sperms through the wall to reach the lumen. A clump of sperm heads (*s.p.*) have pushed in from the distended outer epithelium, and have partly penetrated through the connective tissue-muscle layer of the ovary, which is pushed inwards towards the germinal ridge. Another clump has already passed right through the wall to the lumen (*l.o.*) and has left an open passage (*s.p.p.*) through the connective tissue layer. Many intracellular sperms are seen in the germinal ridge, but the main mass of sperms lies in the ovary lumen outside the region figured. $\times 490$ approx.

FIG. 17—Transverse section of half of an ovarian tube in May. Sperm penetration is now complete. No sperms lie in the outer epithelium or project from the haemocoelic side, and the lumen is distended with a great mass of sperms among which lie a few oogonia or ova. Some sperms lie in vacuoles of the germinal ridge. Two large ova project from the ovary on follicles. The stalks of the follicles are fully formed, flat and one cell thick, and differentiated distally into a darkly staining layer next to the ova. A membrane which stains blue with Mallory has been formed round the ova. Small diverticula of the ovary wall contain sperms from the lumen. $\times 290$ approx.

FIG. 18—Younger follicular ovum from the same ovary. The ovum is of about the same size as in the stage represented in fig. 13. The follicular cells have expanded and pushed the ovum through the connective tissue-muscular layer. The ovum and follicle cells are covered with a thin layer of connective tissue which remains continuous with that of the ovary wall and covers the projecting follicle. No membrane yet covers the ovum. $\times 290$ approx.

FIG. 19—Section of part of the ovary wall in March. All the ova in this ovary have just left the follicles. One ovum is here seen in the ovarian lumen just after it has vacated the follicle (*f.*) which is hollow and communicates (*p.*) with the lumen. The germinal epithelium is a little disorganized by the passage of the ovum. The ovum is still covered by the ovarian egg membrane which will be shed, see text-fig. 7a. $\times 290$ approx.



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